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## A BACTERIAL DISEASE OF ERODIUM AND PELARGONIUM

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WITH PLATE X

### INTRODUCTION

The bacterial disease to be described in this paper was first reported from the vicinity of Austin and San Antonio, Texas, by Heald and Wolf in 1912. These writers described the disease of a cultivated species of Pelargonium very briefly as follows: "Geraniums have been found to suffer in the greenhouse from what is apparently a bacterial spot. The affected leaves show numerous subcircular, brown, or somewhat pellucid areas which are crowded full of bacteria. With the advance of the disease, the intervening leaf tissue turns brown and extensive dead wrinkled areas result which show the original foci as darker spots scattered over the dead portions. Affected leaves generally show more or less chlorosis and may fall before they turn brown. Young plants in the same house were affected with a stem rot which was probably also of bacterial origin." At the time the above brief note was written, the authors of it had not had sufficient opportunity to make an extended investigation into the nature and cause of the disease.

Subsequent investigation by Orlo A. Pratt, a graduate student in the University of Texas, confirmed the statement that the spot is caused by bacteria. Pratt made numerous isolations from varieties of Pelargonium and succeeded in transmitting the disease by inoculations from pure cultures. So far as I am aware, however, his work was not brought to completion and was not published.

During the past year my attention has been directed anew to this problem by the discovery of a bacterial spot on the leaves of *Erodium texanum*, the wild crane's bill, which occurs abundantly in this vicinity. In order to

determine the nature and cause of the leaf spot disease of geraniums, and the relation, if any, existing between it and that of *Erodium texanum*, the present investigation was undertaken.

*Erodium texanum* is native to southwestern Texas. In all of the localities visited it is affected with a bacterial leaf spot which very closely resembles that of the cultivated geraniums, and which has been found to be caused by one and the same organism.

## APPEARANCE OF THE LEAF SPOT

On Erodium, the youngest spots are reddish-brown in color, but very soon change to black and become somewhat watery in appearance. Some of the spots are circular in outline, but they more frequently invade the soft leaf tissue between the veins, and give rise to rather large elongated angular areas which converge toward the base of the leaf (pl. X). These areas may begin at the margin and extend completely to the midrib. The affected tissue finally becomes dry, usually retaining its original color, or with a slightly brownish tinge. The veins are also affected in older stages, and the entire leaf withers and falls. The basal leaves are more vigorously attacked than those which grow above them.

The leaf spot has been observed on four varieties of Pelargonium, and is present in all the greenhouses I have visited. In all of these varieties there is a decided uniformity in the symptoms. The spot first makes its appearance as a minute pellucid dot which can be noted only by holding the leaf in such a manner as to observe it by transmitted light. The spot enlarges with age and soon becomes reddish-brown in the center with a characteristic colorless border. The mature spots are usually subcircular in outline and 2 to 5 mm. in diameter. The dead tissue becomes dry and seared producing a typical "frog eye" spot. The tendency to form large areas between the principal veins which characterizes the disease in Erodium is also manifest in all of the cultivated species observed (pl. X). These areas, however, are not usually so numerous and invariably begin at the margin of the leaf and progress toward the base. It frequently happens that the leaves become chlorotic and fall. That all of the chlorosis and dead areas observed in the infested geranium beds are due to the bacterial invasion is, however, doubtful. Many of the leaves are seriously spotted with the circular spots, but do not reveal any of the larger areas, while in some plants the leaves become chlorotic and show large dead areas which must be attributed to other causes. In all of the affected tissues, particularly in the young areas, bacteria are present in great numbers. A portion of one of the spots macerated in water and stained on the slide reveals the presence of a characteristic organism.

### ISOLATION OF THE ORGANISM

Young spots were selected and immersed momentarily in a solution of bi-chloride of mercury, 1:1000, and then quickly rinsed in sterile water. The tissue was then thoroughly macerated in a drop of sterile water on a flamed slide. The drop of water was then transferred to a tube of plain agar (+ 10), and a series of three dilution plates were poured. The colonies became evident after 48 hours at a temperature of 25°C. The number of contaminations in plates so prepared was uniformly low, and little difficulty was experienced in isolating the causal organism. Isolations were made from Erodium and from four cultivated varieties of geranium.

### INOCULATION EXPERIMENTS

The inoculations were made from cultures isolated from both Erodium and geraniums, agar cultures five days old being used in all cases. These were successful on both hosts when cultures derived from either host were employed. The inoculations were performed by the puncture method as well as by spraying suspensions of the organism upon the leaves.

Punctures were made by means of a sterile teasing needle, and then the organisms were introduced into the opening by means of a platinum needle. The plants were well watered and placed under a bell jar in a dark room, where they remained forty-eight hours. In twenty-four hours from the time of inoculation the tissue adjacent to the opening had become blackened and watery in appearance; in forty-eight hours the spot had enlarged to a diameter of 4 to 5 mm., the tissue being black, watery, and sunken (pl. X). Some of the spots remained circular, but others elongated between the veins, giving rise to the angular spots noted above. The tissue remained black for several days, but eventually became dry, and reddish-brown. It did not fall out to form shot holes, but simulated in all particulars the spots due to natural infection. No distinction whatever could be drawn between the infections resulting from the use of organisms isolated from the same host and those where cross inoculations were made. I am convinced therefore that the organism isolated from Erodium, and that from the cultivated varieties of geranium are one and the same. Little or no difference could be detected in the susceptibility of a wide variety of cultivated forms. The following varieties procured from Vaughan's seed store in Chicago were inoculated: American Beauty, Anna Grothe, Colonel Poirine, Dagata, Jubelpaar, Lucifer, Schöne Ulmerin, Beaute Poitevine, Columbia, Jean Viaud, John Doyle, La Favorite, Mrs. Francis Perkins, S. A. Nutt; Ivy-leaved,—L'Etincelant, Souvenir de Chas. Turner, Beauty of Jersey, Joan of Arc; Variegated-leaved,—Madam Salleroi; Pansy geraniums,—Mrs. Layal.

All of these varieties developed typical spots as noted above. No varieties have been discovered that are immune.

The organism does not seem capable of affecting other species of plants. Inoculations were made into young cabbage plants, lettuce, beans, and nasturtiums. In none of these did the typical blackening occur as with geraniums, and no leaf spots were formed.

## CULTURAL CHARACTERISTICS OF THE ORGANISM

*Agar plates.* Using plain agar, +10 Fuller's scale, the colonies are evident in about 36 hours at a temperature of 25°C. In forty-eight hours the surface colonies are one to two millimeters in diameter in plates that are not crowded. They are whitish, translucent, homogeneous, moist, glistening, slightly raised, with entire margin. With age they become yellowish, especially in the central part, 5 to 6 millimeters in diameter, 3 to 4 zonate, without markings, rather flat viscid, entire. The agar becomes green fluorescent after four or five days. The deep colonies are oval, darker yellow in color.

*Agar stroke.* The growth on peptone agar, +10 Fuller's scale, occurs promptly. In ten to twelve hours there is a fine delicate growth along the needle track which after twenty-four hours becomes 1 to 2 mm. in width. It is whitish, flat, translucent, smooth, moist and glistening. In forty-eight hours the growth is heavy, spreading at base, smooth, cream colored, viscid. The border is whitish, translucent, becoming somewhat scalloped. After three to four days at a temperature of 25°C. the agar has changed to a beautiful yellowish-green. None of the cultures grown in plain peptone agar have failed to produce the green pigment when grown at temperatures less than 30°C. The agar remains yellowish-green, transparent for several days but eventually becomes slightly brownish.

*Glucose agar stab.* The growth in glucose agar stab cultures is evident after 24 hours, but develops much more slowly than in streak cultures. The surface growth is about 2 mm. in diameter, and extends down to a depth of about 1 cm. After three or four days the surface of the agar is completely covered, while the growth along the line of puncture has increased but little, if any. In old cultures the surface growth becomes a deeper yellow then on plain agar. There is no gas produced.

*Lactose agar stab.* Growth in lactose agar stab cultures is very similar to stab cultures in glucose agar. The agar becomes green only in the surface portion.

*Steamed potato cylinders.* In twenty-four hours the growth on potato cylinders is yellowish, translucent, spreading somewhat from the needle track; in forty-eight hours there is a copious, moist, glistening, cream-colored

slime; after four or five days the growth has covered the entire sloped surface of the potato. The medium becomes a grayish brown in forty-eight hours and this is followed by the green fluorescent pigment characteristic of the agar cultures. On some potatoes the green pigment is not so prominent. Old potato cylinders on which the organism has grown for several weeks remain firm and resistant. The starch in such tubes gives a violet reaction with iodine.

*Steamed cocoanut.* Pieces of cocoanut partially submerged in distilled water were inoculated both on the surface and in the water. The growth takes place promptly, and after forty-eight hours the entire surface becomes covered with a very copious yellow slime. The water is densely cloudy. The green pigment is not produced in this medium.

*Litmus milk.* In milk colored a deep blue with litmus there is a watery, almost transparent band about 2 to 4 mm. in width after forty-eight hours. This is followed by a zone several shades lighter in color than the original medium extending about one-half the depth of the medium, while the bottom layer shows no change. The lines of demarcation in these three zones are sharply defined. No coagulation of the milk takes place. After four or five days the middle zone has extended to the bottom of the tube, but is somewhat lighter in the upper portion. A rather dense precipitate is present at the bottom, and a flocculent precipitate occupies the upper portion to a depth of one centimeter. The cream ring is yellowish. In eight days all of the original color has disappeared, and the liquid is now clear yellowish with a precipitate at the bottom and some floating particles on the surface. The color returns to the zone about 1 cm. from the surface in old cultures. The medium becomes quite viscid with age.

*Sterile milk.* In forty-eight hours there is a clear transparent ring of whey to a depth of about 2 mm. below the cream ring. This band increases in width to 4 mm. in seventy-two hours, and the entire tube has become somewhat clear and watery in appearance. In five days the tube is almost clear transparent with a heavy whitish precipitate at the bottom, and some larger particles float on the surface. There is no coagulation of the milk, but a gradual separation and digestion of the casein. The culture becomes green with age.

*Dunham's solution.* Growth in Dunham's solution appears promptly. In twenty-four hours the medium is slightly turbid. In forty-eight hours there is increased cloudiness with numerous small zoöglea in the upper portion. With age, a fine delicate membranous surface growth forms which is easily broken by shaking the tube. The growth is less copious than in peptone bouillon. The green pigment does not form in this medium.

*Peptone bouillon.* In peptone beef bouillon without NaCl reaction +

10 Fuller's scale, there is a uniform rather dense cloudiness in twenty-four hours. In forty-eight hours there is an abundant growth throughout the tube. A delicate membrane attaches to the wall of the tube at the surface, but there is less tendency to form zoöglea in the surface layer than in Dunham's solution. This medium becomes somewhat green after three or four days.

*Dunham's solution with methylene blue.* The growth in this medium does not appear so promptly. The methylene blue is not reduced. Tubes were kept under observation for a period of four weeks, but with no evident change in color of the blue.

*Bouillon with sodium chlorid.* In neutral peptone bouillon containing 2.5 per cent NaCl, growth is somewhat retarded, but occurs within four days. Growth does not occur in tubes to which 5 per cent NaCl is added.

*Bouillon over chloroform.* Growth in tubes containing 10 cc. of peptone bouillon over 5 cc. chloroform is retarded but develops normally after forty-eight hours. The growth is limited to the upper part of the tube; numerous fragile zoöglea are formed at the surface. The green pigment is developed as in all bouillon cultures.

*Decoction of Erodium leaves.* A decoction made by boiling 50 grams of Erodium leaves in one litre of water strongly retards the growth. There is only a slight clouding in this medium.

*Erodium agar.* To some of the above decoction 1.5 per cent agar was added. Growth is retarded and never more than a feeble streak is produced.

*Decoction of geranium leaves.* A decoction of geranium leaves made up as above strongly retarded growth, as did also the agar prepared from it.

*Nitrate bouillon.* In bouillon containing 0.3 per cent potassium nitrate and 1 per cent glucose growth occurs promptly and is copious after three or four days. A membrane develops at the surface which does not fall apart upon shaking.

*Fermis' solution.* Fermis' solution is somewhat clouded in twenty-four hours. A thin scum forms on the surface somewhat as in the nitrate bouillon. The culture becomes green fluorescent with age.

*Cohn's solution.* Growth in Cohn's solution is strongly retarded but occurs after four days in tubes which are heavily inoculated. There is no pigment produced in this medium. The surface zoöglea form in old cultures.

*Uschinsky's solution.* The growth occurs in this medium within twenty-four hours and develops as in peptone bouillon. A fragile zoögleal scum covers the surface in old cultures. The green pigment is not developed.

*Gelatin stab.* In peptone gelatin (+10) stab culture the growth is visible in forty-eight hours. Liquefaction occurs promptly. In cultures three days old there is a cup-shaped depression about 4 mm. in depth, but growth has not taken place along the line of the stab to a depth of more

than one centimeter. The liquefaction proceeds rather slowly. In cultures eight days old there is a layer about one centimeter in depth which is liquefied. Complete liquefaction occurs in four weeks.

*Lactose litmus agar.* Growth proceeds in this medium without retardation. The medium is reddened in old cultures.

*Steamed carrot cylinders.* Growth is copious on this medium. The entire surface of the cylinder is covered after three or four days with a wet, smooth glistening slime. The medium becomes somewhat brown in color.

*Steamed white turnips.* This medium is favorable for the growth of the organism. The streak is well developed in twenty-four hours and quickly spreads over the entire surface. It is wet, glistening whitish in color. The tissue becomes softened and brown.

### OTHER CHARACTERISTICS

*Production of indol.* Indol is produced in cultures fourteen days old, but is not present in young cultures. The organism was grown in Dunham's solution and was tested by means of sulphuric acid and sodium nitrite.

*Hydrogen sulphid.* No hydrogen sulphid is produced in any of the media tested. The organism was grown in peptone bouillon for several days. Strips of filter paper moistened with lead acetate were then suspended in the tube and held in position by the cotton plug. Similar tests were made with peptone beef agar, lactose agar, and potato cylinders. No blackening of the paper could be detected after prolonged exposure.

*Ammonia production.* Ammonia is produced in peptone beef bouillon. Tests were made on cultures ten days old. Filter paper moistened with Nessler's solution and suspended in the tubes becomes brownish-red upon heating the tube over a water bath.

*Nitrate reduction.* Nitrites are not formed from nitrates. The organism was grown in nitrate broth to which 1 per cent glucose was added. The growth in this medium is luxuriant. Tests were made after ten days cultivation. The starch-iodine method and the napthylamine-hydrochloride method both gave negative results, while *Bacillus coli* grown in the same medium was decidedly positive in its reaction.

*Reduction of litmus.* Litmus is reduced in from six to eight days in sterile milk.

*Methylene blue.* The tests for reduction of methylene blue were negative.

### THERMAL RELATIONSHIPS

*Thermal death point.* The thermal death point was tested for bouillon cultures which had grown for twenty-four hours and for six days. Thin-walled test tubes containing exactly 10 cc. of bouillon (+10) were inoculated

with two 2 mm. loops from the culture to be tested. The tube was thrust into a large water bath the temperature of which was kept constant for the ten-minute periods. The twenty-four-hour cultures succumb between 48° and 49°C. The thermal death point under the above conditions is given therefore as 48.5°C. No effort was made to determine the fractional part of the degree. The six-day cultures survived a temperature of 53°C. for ten minutes.

*Optimum temperature.* Cultures were grown under a wide range of temperatures. The apparatus was not at hand, however, to keep so wide a range of constant temperatures as was desirable. The cultures grown at 25° to 27° were more luxuriant than at 30° to 34° or 20° to 23°. The optimum temperature is therefore about 27°C.

*Minimum temperature.* The organism grows well at low temperatures. At 8° agar cultures make a typical growth, with production of pigment on slant agar. Growth takes place in both agar and bouillon at a temperature of 0°C., but does not take place at −1°C. Cultures kept at −4°C. three days remain alive and develop typically when restored to a temperature of 25°C.

*Maximum temperature.* Growth does not occur at 35° in either bouillon or agar.

### TEST FOR ANAEROBISM

The test for anaerobism was carried out by the U-tube method as proposed by Erwin F. Smith. Two small test tubes containing peptone bouillon were inoculated heavily from a bouillon culture forty-eight hours old. They were placed in one arm of the U-tube which was then stoppered with a rubber stopper and stood in a beaker of glycerin. A sufficient quantity of sodium pyrogallate in a large test tube was then placed in the other arm which was quickly thrust into a beaker of mercury. The oxygen was absorbed promptly. This culture remained eight days when the tubes were removed and examined. There was no trace of cloudiness in either of the cultures. The experiment was repeated three times but in no case did growth take place, even with a prolongation of time to fifteen days. In each instance the cultures became cloudy after removal from the anaerobic tube and incubation at room temperature. The organism will not therefore grow in an atmosphere devoid of oxygen, but will retain its vitality in an atmosphere of nitrogen.

### RELATION TO MOISTURE

The organism does not withstand long periods of drying. Bouillon cultures six days old were employed in making this test. Glass rods were placed through the cotton stoppers of test tubes and then sterilized in the

hot air sterilizer. The rods were then dipped into the culture to a uniform depth, returned to the tubes and allowed to dry at room temperature 18° to 22°C. At intervals of twenty-four hours one of these rods was transferred to sterile bouillon. Growth takes place up to six days, after which all of the tubes remain sterile. Younger cultures were not tested. It is reasonable to suppose, however, that the period which desiccation can be withstood is shorter in young cultures.

### GROWTH IN FERMENTATION TUBES

The organism has been grown in 2 per cent peptone water plus one per cent each of the following carbon compounds: saccharose, dextrose, lactose, maltose, and glycerin. No gas formed in any of the fermentation tubes. The growth is heavy in the open arm of the tubes, and the outer part of the U-tube, but does not occur in the closed arm. The line of demarcation remained sharply defined throughout the experiment. Little or no difference could be detected in the amount or character of the growth in the various compounds tested.

The cultures were tested for acidity after they had grown 21 days. In maltose, the acidity increased from +8 to +12; in saccharose from +10 to +18; in glycerin from +10 to +16; in glucose from +10 to +17; in lactose from +8 to +16. The organism produces acid in all of the compounds in which it was tested, but does not produce gas from any of them.

### SENSITIVENSSS TO ACIDS AND ALKALIES

The organism grows best in culture media that are acidulated to +15 or +20 with HCl. Growth does not occur at +40 and is retarded above +30. The same degree of acidity is not tolerated when malic, tartaric or lactic acids are employed. The growth in +8.5 lactic acid appears to be about the optimum for this acid, and the maximum is lower than for hydrochloric. Growth fails at +30 tartaric and malic.

In media titrated to −10 with normal NaOH the growth is retarded, while no growth occurs at −20. In neutral bouillon growth proceeds more slowly than in +10 to +20.

### MORPHOLOGY OF THE ORGANISM

The organism is a rather short rod, with rounded ends, occurring usually singly or in pairs, but sometimes in short chains in liquid media (pl. X). Organisms from a forty-eight hour slant agar culture stained with aniline-gentian violet are 1.2 to 1.8$\mu$ in length and 0.6 to 0.8$\mu$ in diameter.

No spores have been observed in the oldest cultures examined. Cultures which had grown two months were stained and examined for spores, but in all instances the results were negative. These same cultures succumb to a temperature of 80°C. in ten minutes. Lower temperatures were not tested.

The organism is very actively motile by means of polar flagella. Young agar cultures were stained by Loeffler's and Van Ermengem's methods. The best results were secured by Loeffler's method. The flagella are present at only one pole, and are one to three in number. They are rather long, wavy, fine and delicate, and stain with some difficulty. Involution forms, if present, are rare even in old cultures. Numerous stains made from some of the old liquid cultures have established this fact. Occasionally forms have been observed that are larger and more vacuolate than the ordinary cells, but typical involution forms have not been observed.

## NATURAL INFECTION AND CONTROL

In all of the greenhouses I have visited in both Austin and San Antonio the leaf spot is very abundant. It is more prevalent in crowded beds where the plants remain moist and the light is not so dense. The infection takes place where no insect injury is visible. I am convinced that watering by sprinkling the plant is the chief way in which the infection is spread. It seems reasonable to suppose that the primary infection comes from the native Erodium, but as geraniums have not been examined from other sections of the country where this plant does not occur, I cannot draw such a definite conclusion. I have not been able to examine other wild species of the order to determine whether or not they are infected. It is hoped that the present investigation may cause observations to be made elsewhere which will more definitely determine the range of the disease. Under greenhouse conditions the leaf spot should be readily controlled by the removal of the diseased leaves, and by careful attention to the method of watering.

## TECHNICAL DESCRIPTION OF THE ORGANISM

The organism does not appear to agree in all particulars with any known organism, although it has many characteristics in common with the yellow fluorescent group of plant parasites.

**Bacterium**[1] **(Pseudomonas) Erodii** n. sp.

According to the numerical designation of the Society of American Bacteriologists the group number of *Bacterium Erodii* is 211.2222122. Form, a short, rather plump rod with rounded ends, borne singly, in pairs or in very short chains. Very actively motile by means of polar flagella. Involution forms rare; no spores or capsules distinguished; produces green fluorescent pigment; liquefies gelatin; reduces litmus; produces ammonia and indol; does not reduce nitrates to nitrites; does not produce hydrogen sulphid; grows in Uschinsky's, Fermis', and Cohn's solutions; separates the casein from milk and gradually digests it; obligate aerobic; optimum temperature 27°, minimum temperature − 1°C., maximum temperature 35°C.; thermal death point 48.5°C.; resists desiccation six days; stains with basic aniline dyes; not acid fast; negative to Gram's method; grows best in media titrated to +15 Fuller's scale; no growth above +35; no growth at −20; pathogenic to *Erodium texanum* and a large number of cultivated varieties of geraniums.

UNIVERISTY OF TEXAS
SCHOOL OF BOTANY

[1] The generic name Bacterium is in accord with the usage favored by E. F. Smith (Bact. in Rel. Pl. Dis. **1**: 171). By Migula's system the name becomes Pseudomonas Erodii.

EXPLANATION OF PLATE X.

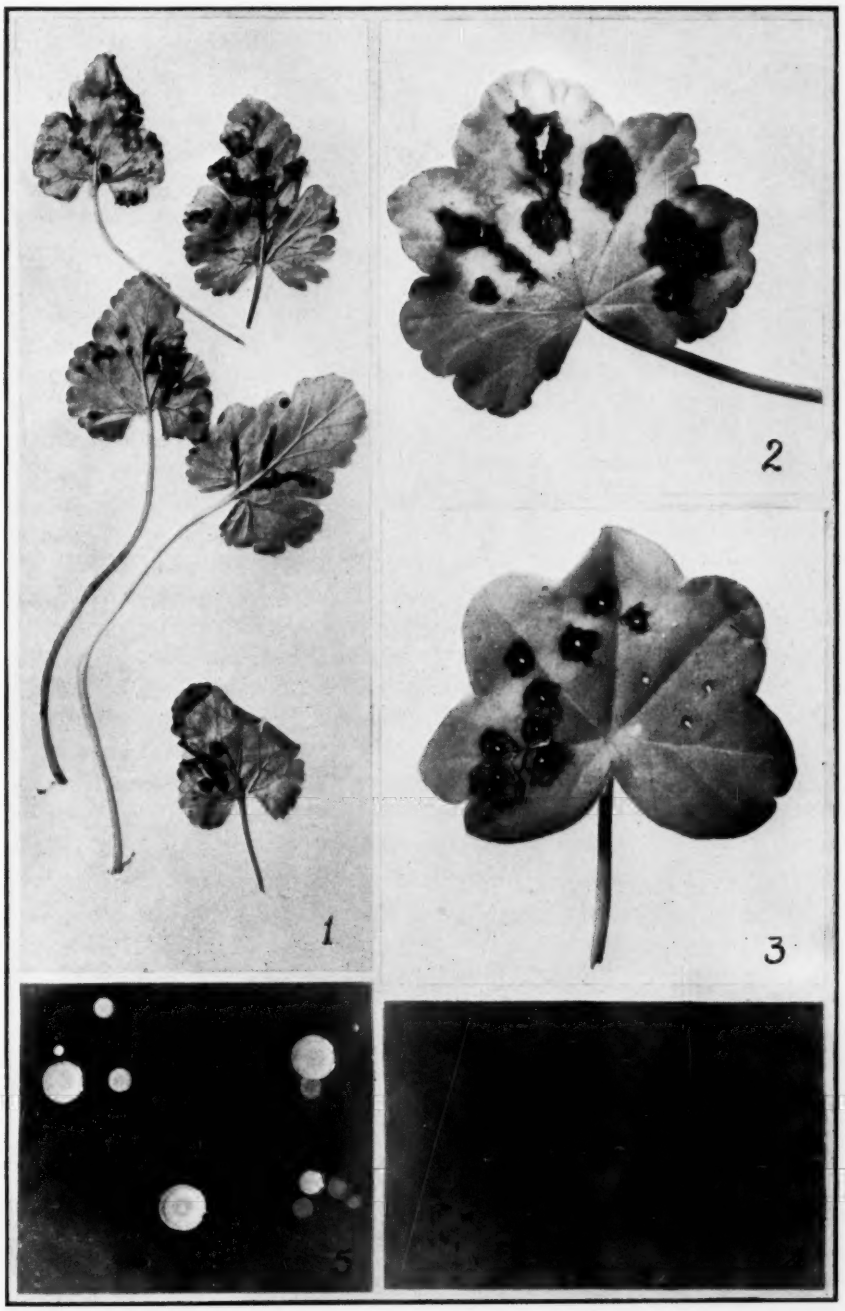
FIG. 1. Leaves of Erodium texanum showing natural infection as it occurs on the leaves in the field. The spots of this type develop throughout the winter. Photograph taken from leaves collected February 2.

FIG. 2. Leaf of geranium inoculated with pure culture of Bacterium Erodii isolated from Erodium texanum. The spots are five days old.

FIG. 3. Leaf of geranium inoculated with B. Erodii and with some of the punctures not inoculated. Photograph four days after inoculation. The spots reach this size within forty-eight hours, but are more water-soaked and not so black in color.

Fig. 4. Photograph from pure culture grown on nutrient agar twenty-four hours, and stained with aqueous gentian violet. Photographed with Zeiss 2 mm. apochromatic objective, and ocular 8.

FIG. 5. Colonies ten days old on plain peptone beef agar.




LEWIS: A BACTERIAL DISEASE

# FIELD STUDIES ON THE ENDOTHIA CANKER OF CHESTNUT IN NEW YORK STATE

W. H. RANKIN

WITH PLATE XI AND TWO FIGURES IN THE TEXT

The experimental work herein reported was conducted in temporary field laboratories located at Highland, New York, in the summer of 1911, and at Napanoch, New York, in 1912 and 1913.[1] The data concerning observations and the distribution of the disease in New York State were gathered during the surveys conducted for that purpose in the summers of 1911 and 1913.

At the time of the beginning of this work but little experimental data were available concerning the life-cycle of the pathogen in the forest. The urgent need of this data was realized at that time when public agitation demanded adequate control methods. So many points vital to the feasibility of any control method suggested were lacking that many pathologists refrained from endorsing these measures until further details concerning the life habits of the fungus were obtainable. With these points in view the field work was started in 1911. In the spring of 1912, Mr. P. J. Anderson started a series of field investigations in Pennsylvania. Any similarity of working plans and methods followed between the work of Mr. Anderson and that of the writer is explainable through the fact that they were constantly associated in the work and freely exchanged their views and results.

[1] These investigations were suggested by Prof. H. H. Whetzel. To him and to Prof. Donald Reddick the writer is indebted for many valuable suggestions concerning details of manipulation and for aid in the preparation of the manuscript. The writer also wishes to thank the following for many services rendered which have facilitated the work: To Dr. Haven Metcalf for his personal attention, for the use of his laboratory at Washington, D. C., in April, 1911, and for the financing of the two surveys in 1911 and 1913; to Mr. C. R. Pettis and Mr. G. G. Atwood for furnishing assistants for the surveys; to the Conservation Commission for time allowed to carry on this work while in their employ as State Forest Pathologist during 1912 and 1913; and to Mr. George Deyo, Assistant Superintendent of Reformatories for furnishing trees on the Napanoch Reformatory lands and for many personal services.

## DISTRIBUTION OF ENDOTHIA CANKER IN NEW YORK STATE

A survey of the conditions existing in the state was the first step necessary to preface a consideration of any state-wide control measures. At the suggestion of Dr. Metcalf this work was begun in April, 1911, by the writer, working as Special Agent of the Department of Agriculture. The State Conservation Commission and the State Department of Agriculture became identified with the movement by furnishing assistants. The aim was to scout as rapidly as possible north and west of the lower Hudson River district where it was known that the disease already existed.

At the outset of this investigation, the information available concerning the distribution of the disease attributed the northern limit of its range to southern Dutchess and central Orange Counties. It was known to be generally established in the western part of Long Island, Staten Island, and the counties of New York, Westchester, and Rockland. It was also expected that Orange County had been invaded to some extent.

No accurate information was available as to the exact range of the chestnut in New York. Four thousand circular letters were mailed to correspondents of the Conservation Commission and, on the basis of the replies, a map was made which has since proven accurate. Reference to the accompanying map (fig. 1) will show that the chestnut area of the state lies in two almost separated areas, the Hudson River Valley, and the central and western parts of the state. The two areas are connected by a narrow band of chestnut skirting the Delaware River in Sullivan and Delaware Counties. There is no chestnut to be found in the Adirondack Mountains and but little, and for the most part none, in the Catskill Mountains and the highlands of Ulster, Sullivan and Delaware Counties. The extreme northeastern corner of the state contains but few scattering chestnut trees.

### *Conditions in 1911*

The map (fig. 1) shows the results of the survey of the summer of 1911 in fixing the limits of spread of the disease. East of the Hudson River the disease was found abundantly north as far as Albany. The chestnut is most abundant along the state line between New York and Connecticut and Massachusetts in the range of mountains making up the Berkshires. Along the Hudson River the chestnut is plentiful but not so abundant as in the hills. It was along the state line in the heavy stands of chestnut that the disease had made the greatest ravages. However, the disease was present in every locality where there was any quantity of chestnut. Practically every town east of the Hudson River is represented in the list of those in which the disease was found. In the few

towns in this region from which the disease was not reported the stand of chestnut was slight. In the central part of Rensselaer County there is little chestnut; especially is this true near the Hudson River. Back along the state line separating New York from Massachusetts and Vermont in the continuation of the Berkshires north there is considerable chestnut but no disease was found there. The natural northern limit of the chestnut as will be seen by the map is in the towns of Fort Ann and Granville in Washington County. The disease was found very abundant throughout central Washington County where many lumbermen were cutting chestnut as fast as possible in the towns of Hartford, Argyle, and Hebron to keep ahead of the disease.

A reinspection of the area in Rensselaer, Albany, Saratoga, and Washington Counties where no spot infections were found in 1911 would very probably show the disease present now. The numerous spot infections in central Washington County indicate an early center of infection which was spreading southward and would soon join with the main line of advance up the Hudson River Valley.

West of the Hudson River the disease had not advanced as far north. Practically the entire stand of chestnut in Orange and southern Ulster Counties was diseased. In the Catskill Mountains proper there is much chestnut in the valleys opening into the Hudson River Valley. The disease had spread up the southernmost of these valleys as far as there is chestnut to be found, but in the Esopus Valley and those north of it the disease had spread only a short distance up the valleys. In Albany County the chestnut is scattering except in the Helderberg Mountains and it is very scarce in the western part. A complete survey of Albany County was not made, owing to the unimportance of this information. However, the disease was found to be general in the southern part of the county along the Hudson River.

The connecting link between the chestnut region of the Hudson River Valley and that of the central and western part of the state, is the strip in Sullivan County along the Delaware River. Chestnut is abundant here for fifteen to twenty miles away from the river. Many isolated spot infections were found here. General spread extended about as far up the Delaware River as Port Jervis. A few spot infections were found in southern Delaware County along the Delaware and Susquehanna Rivers. One spot infection was found at Masonville, Delaware County, and was destroyed. No infections were known west of this region in 1911. A special investigation of the cause of the dying of the chestnut near Cooperstown, Otsego County, was made but it was found that it was not due to the canker.

The following is a brief summary of the report made to Dr. Metcalf

as to the advisability of attempting the control of this disease in New York State, by the cutting-out method.[2] The results of the survey prove that the disease has a greater foothold in New York State than was expected. On account of the vast area that is now affected, the problem presents many difficulties. The chestnut left in the state outside of the line of advance is of considerable value and worth saving if it were possible to do so economically. It is well, however, to consider the difficulties involved. The advancing line is in a region in which the topography is extremely rough, the hills thickly wooded and the chestnut abundant. Under such adverse conditions it would be, to say the least, a gigantic task if not indeed an impossible one. If natural barriers were more favorable, such a scheme might be more feasible. More recent developments concerning the method of spread of the ascospores by the wind only lend further argument against the practicability of the cutting-out method under New York conditions.

### *Conditions in 1913*

A hasty resurvey of the territory bordering on the line of advance determined in 1911 was made in September, 1913, for Dr. Metcalf. The area just west of the "chestnut-free" belt was gone over and the line as drawn on the map (fig. 1) was fixed as representing approximately the limit of spot infections at that time. The strip along the Delaware River was found to be abundantly affected now where only isolated spots were found in 1911. The disease does not seem to be spreading as rapidly north as west and no infections in Otsego County were found, suggesting the value of the natural barrier formed by the "chestnut-free" belt in the Catskills. This belt composed of mountainous land, 1000 to 3000 feet above sea-level varies from 30 to 40 miles in width. Apparently this distance has been sufficient to protect the chestnut in Otsego County from infection by spores carried westward from the diseased trees in the Hudson River Valley.

## PATHOGENICITY

Murrill[3] proved that *Endothia parasitica* (Murr.) Anders. is pathogenic on *Castanea dentata*, the American chestnut. It is so easy of demonstration that it has never been questioned since that time. Constant association of the fungus with the canker has been readily noticed by all

[2] Metcalf, H. and Collins, J. F. The control of the chestnut bark disease. U. S. Dept. Agr., Farmers' Bul. 467: 1–24. 1911.

[3] Murrill, W. A. A serious chestnut disease. Journ. N. Y. Bot. Garden **7**: 143–153. 1906.

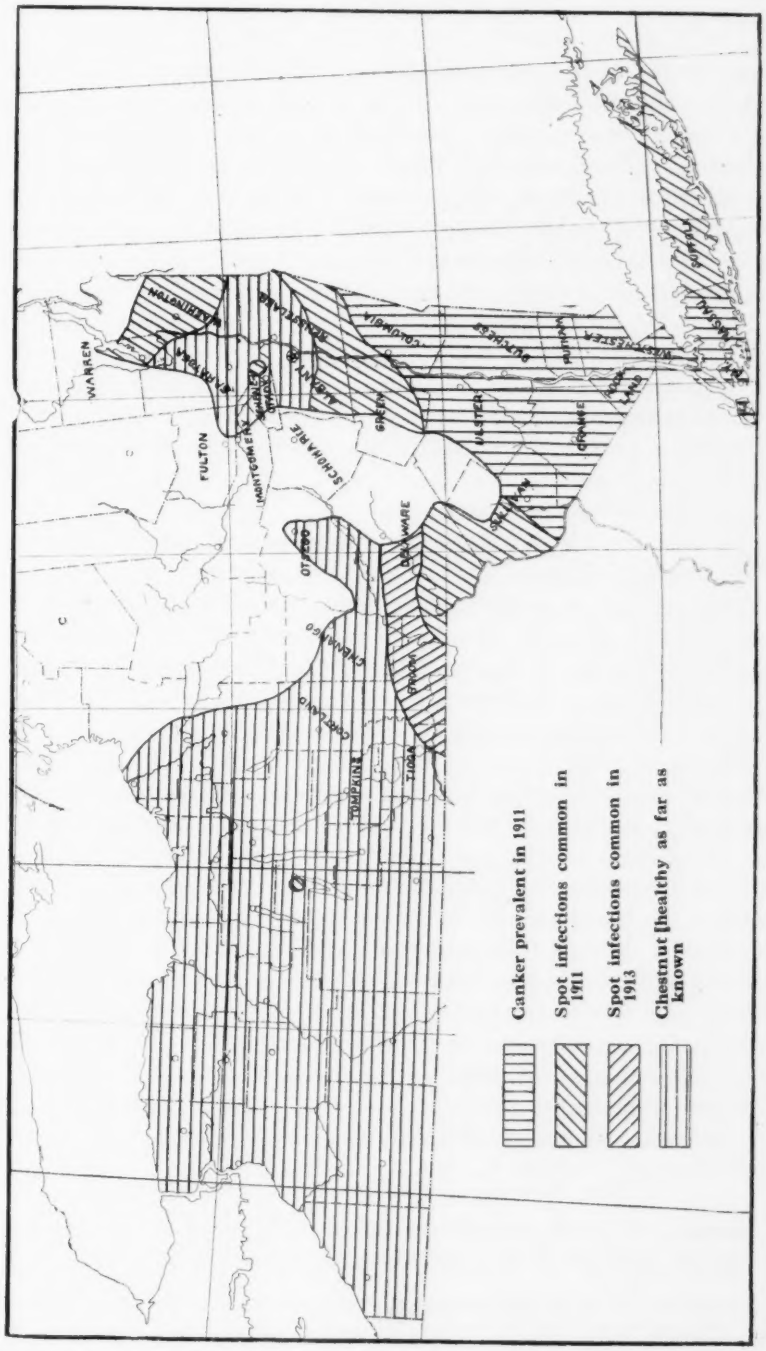



FIG. 1. Map of New York State showing the distribution of the Endothia canker as determined by surveys made in 1911 and 1913.

writers. It has been isolated and grown in pure cultures by a number of pathologists. Inoculations from pure cultures and the production of typical cankers were reported first by Murrill (loc. cit.) and later by various others. The writer has carried out Koch's four "rules of proof" many times in connection with the experimental work here reported.

Inoculations with the fungus isolated from chestnut were made on *Quercus prinus*, *Q. rubra*, *Q. alba*, and *Q. coccinea*. Out of seventy-one inoculations made in slits in the bark of *Quercus prinus* only eight showed any advance of the mycelium into living tissues. In no case were typical cankers formed and the advance into living tissues was only at a certain place, usually at the side of the injury. On *Q. coccinea* two inoculations out of thirty-five showed the mycelium advancing in healthy tissue. A single inoculation in the bark of *Q. alba* developed fans of mycelium which spread in the living bark for about a centimeter. Four inoculations on *Q. rubra* gave entirely negative results.

In all cases the fungus grew in the injured bark and produced pycnidia and spore-horns. In the cases mentioned where slight advance was made into healthy tissues, typical mycelial-fans were produced as in the chestnut so that one could say the fungus was parasitic.

The writer[4] made an examination of the chestnut in the vicinity of Lynchburg, Virginia, in February, 1912, and collected abundant material of the saprophytic fungus which later was proved to be *Endothia radicalis* (Schw.) De Not. At that time although differences from *E. parasitica* were noted in the asci and spore measurements it was thought that a collection made at that time of year might account for those variations. Inoculations, however, which were begun in May, 1912, soon proved the individuality of this fungus since in no case could infection be secured on the chestnut. Sixty inoculations were made on the chestnut by inserting mycelium from pure culture in slits in the bark, with negative results in all cases. Likewise, twenty-one inoculations on *Quercus prinus*, ten on *Q. coccinea* and two on *Q. rubra* gave negative results. In all cases the fungus invaded the tissues injured in making the inoculation and pycnidia and spore-horns were produced abundantly, but in no case did the mycelium enter uninjured bark and establish pathologic relations with the host. No mycelial-fans are formed by this fungus.

### INOCULATION AND INFECTION EXPERIMENTS

It seemed desirable to attempt the inoculation of uninjured parts of the host to ascertain if the natural cracks in the rough bark or the lenti-

[4] Rankin, W. H. How further research may increase the efficiency of the control of the chestnut bark disease. Pennsylvania Chestnut Blight Conference. Stenographic Rept., pp. 46–48. 1912.

cels were possible infection courts. Microscopic examination of sections through the lenticels in the spring showed that practically no suberization of the cells at the base had taken place, and apparently the phellogen layers were exposed to infection. Likewise, the broad cracks in the bark resulting from the rapid growth in the spring and early summer would suggest that infection could be possible at these seemingly unprotected places. During the summers of 1912 and 1913, several hundred inoculations of different types were made with these points in mind.

### *Inoculation of lenticels*

In order to maintain a proper humidity for spore germination on the bark, glass rings were affixed to the branches with grafting wax and then after spraying the bark thus inclosed with a suspension of ascospores in water, a cover-glass was sealed over the ring. Moisture was retained perfectly for two or three weeks in these rings.

Eleven such inoculations were made July 19, 1912, on the bark of trees less than ten years old, using ascospores in water. In no case was infection accomplished.

Twenty-eight similar inoculations were made on April 16, 1913, using ascospores in water. The results were negative. Twenty-two checks made by inoculating the same material into wounds in the bark gave ten cankers.

Again on July 25, ten more inoculations of the same type were made but no infections were obtained. An equal number of check inoculations into wounds all produced cankers.

These negative results under conditions which were evidently conducive to infection, were it possible, seem to prove that infection cannot be attributed in any great percentage of cases to lenticel infection.

### *Inoculation of the natural cracks in bark*

From early spring until mid-summer the rapid growth made by the chestnut causes wide cracks to open up at the bottom of the crevices in the bark. Microscopic examination of cross sections of such cracks shows that the light colored tissues which are exposed at the base of these cracks are not suberized.

On June 11, 1912, ascospores in water were sprayed into three natural cracks in the bark of an old tree. Infection was secured in the three cracks.

On July 19, six inoculations were made by affixing glass rings over natural cracks which showed deep slitting. This was done in the same

manner as in the lenticel inoculations. Ascospores in water were used as the inoculum. The results were negative.

More carefully performed experiments were made in the summer of 1913 to establish this point. On April 17, fourteen rings were affixed over deep cracks in the bark and ascospores used as the inoculum. All gave negative results. Twenty-eight inoculations with the same material made in wounds in bark gave ten cankers.

On June 11, 1913, fifty-six inoculations were made by spraying ascospores into natural cracks and leaving them unprotected. The ascospores remain viable for some time as will be shown later, and, when natural conditions were correct for spore germination, infection should result if this were possible. This experiment duplicated in almost all points the natural conditions of inoculation with wind-blown ascospores. However, all these inoculations gave negative results although the same material sprayed into twenty wounds in the bark on neighboring trees produced eighteen cankers.

On June 16, twenty pieces of bark containing mature perithecia were affixed to trees so that during favorable weather conditions ascospores would be ejected into newly-formed cracks in the bark. The ascospore material used was previously tested. In no case did infection result from these inoculations, which certainly duplicated perfectly natural conditions.

On June 17, one hundred and thirty-one inoculations were made like those of June 11, by spraying ascospore material into natural cracks and leaving them unprotected. Out of these inoculations only one infection occurred. Careful examination did not show a wound of any character and the reason for success in this one and not the others is unexplainable.

From these experiments it seems to be proved that the natural cracks in the bark, although apparently exposing unprotected tissues, are not a common infection court for the fungus, and that the tree is not naturally predisposed because of these seemingly morphological defects in the bark. When these results are compared with those following, in which wounds were made in the bark, it is reasonable to draw the conclusion that the fungus is not able to penetrate healthy tissue as single hyphae, but when sufficient dead tissue is present to afford the fungus a chance to form the mycelial-fans it invades the living tissues.

### *Artificial inoculation of injured parts of host*

Numerous inoculations were made in 1912 in which various types of wounds were made in the bark of branches more than three years old in which different sorts of inoculating material were used. In table I are summarized the results of these inoculations. The slits in the bark were

made by inserting the point of a sharp knife in a tangential direction under the outer layers of the bark, and then by lifting the tissue slightly the inoculum was sprayed or placed in the wound. A small area of the bark was killed and thus ideal saprophytic conditions were furnished for the rapid development of the fungus and its subsequent invasion of living tissues. For comparison, to obviate this wounding of the tissue, injections were made by using a hypodermic syringe. The needle was inserted lengthwise of the stem to a depth of about an inch. A very small amount of the tissue along the canal was killed. The lower percentage of successful infections obtained was probably due to the fact that much less favorable saprophytic conditions were available for the initial growth of the fungus. Still another method of inoculation was tried in which a hand saw was used to prune off diseased limbs, cutting through a canker, and then used to prune healthy limbs. The stubs were left unprotected. The relatively high percentage of successful infections points to the chance of such inoculations being possible where attempts are being made to eradicate the disease from shade trees.

Mycelium from pure cultures, bits of affected bark, conidial spore-horns, pycnidia from wood, and conidia and ascospores suspended in water were used in making inoculations. In the period of time covered by table I

TABLE I

*Results of inoculations made in wounds, May 1 to September 1, 1912*

| METHOD | INOCULUM | NUMBER MADE | NUMBER INFECTED | PERCENTAGE INFECTION | AVERAGE PERCENTAGE INFECTION |
|---|---|---|---|---|---|
| Slits in bark | Ascospores in water | 89 | 88 | 99 | 93 |
| | Conidia in water | 116 | 95 | 82 | |
| | Mycelium from cultures | 61 | 61 | 100 | |
| | Diseased bark | 93 | 93 | 100 | |
| | Conidia dry | 10 | 10 | 100 | |
| | Pycnidia from wood | 11 | 4 | 36 | |
| Injections | Ascospores | 107 | 74 | 69 | 73 |
| | Conidia | 56 | 46 | 82 | |
| Saw wounds | Diseased bark | 50 | 26 | 52 | 52 |

it will be seen that all these forms of inoculum were capable of producing infection.

During the summer of 1911 several inoculations were made in which mycelium was placed in insect tunnels. Some were protected by binding moist cotton over them while others were left unprotected. In no case

was infection secured. It seems that such injuries are at least an uncommon infection court.

From the foregoing results of inoculations made in injured and uninjured tissue and the results of other writers, the conclusion is drawn that the fungus is strictly a wound parasite. The opinion expressed by Smith[5] with reference to the use of the term wound parasite and mass action is especially interesting in this connection. Wounded tissue and a brief saprophytic growth, sufficient to produce mycelial-fans are necessary before living tissues can be invaded and infection accomplished. The fact that infection cannot be obtained in old wounds, such as insect tunnels, is unexplained as yet. It may be possible that partial callus development and suberization of the exposed tissue accounts for this fact. Infection may frequently result from ascospores and conidia washed down the tree into insect tunnels when they are newly made.

### *Natural inoculation*

No credence can be placed in observations, made after natural inoculation, as to the method of entrance of the fungus. In the stages of development where such observations might be depended upon, the cankers are as yet unnoticeable. The early cracking and decomposition of the bark to a pulverulent and stringy mass in most cases soon obliterates the facts necessary for accurate data. However, broken twigs and the stubs of limbs are often found with cankers developing around them. As a general rule it has been the writer's observation that the majority of primary infections of healthy trees take place in the smaller branches and usually in limbs toward the top or outside of the crown where they are more or less exposed.

To ascertain with what uniformity certain factors concerning natural inoculation really occurred, tabulations were made with reference to primary infections.

As to the height of primary cankers on trees the following figures were obtained:

| | | | |
|---|---|---|---|
| At base | 14 | On trunk | 77 |
| Below 10 feet | 89 | On exposed branches | 81 |
| Above 10 feet | 76 | On unexposed branches | 24 |

Tabulations as to the orientation of primary cankers yielded results which show that there is no constancy in this factor:

| | | | |
|---|---|---|---|
| North | 30 | East | 30 |
| South | 26 | West | 23 |

[5] Smith, E. F. Bacteria in relation to plant diseases. **2**: 36-39, 71. 1911.

*Relation of condition of host to relative susceptibility*

The condition of the host has been held by some writers to be the primary factor accounting for the extreme aggressiveness of this fungus. Clinton[6,7,8] has been the chief exponent of these views. At the same time it must be borne in mind that the belief that the pathogen had existed in this country heretofore as an overlooked saprophyte has caused many to overestimate the purely observational or theoretical foundations for this belief. The finding of *Endothia parasitica* in China where it apparently is a native species, and the results of numerous inoculation experiments, leave but little support for the belief that an abnormal condition of the host has accounted for its susceptibility.

To ascertain the susceptibility of first, second, and third year twigs, inoculations were made by spraying ascospores into slight wounds made with the point of a scalpel. It is evident from table II that second and

TABLE II

*Results of inoculations of young growth*

| DATE AND MATERIAL | FIRST YEAR GROWTH | | SECOND YEAR GROWTH | | THIRD YEAR GROWTH | |
|---|---|---|---|---|---|---|
| | Inoculations made | Infections secured | Inoculations made | Infections secured | Inoculations made | Infections secured |
| June 11, 1911, mycelium.. | 10 | 0 | 10 | 10 | 10 | 10 |
| July 19, 1912, ascospores.. | 15 | 0 | 15 | 6 | 15 | 9 |
| July 20, 1912, ascopores... | 25 | 1 | 10 | 6 | | |
| July 22, 1913, ascospores.. | 6 | 5 | 5 | 5 | 4 | 4 |

third year's growth are susceptible at all times, while first year's growth, which is seemingly immune in spring and early summer, can be infected later in the summer. This is in accord with observations of natural infection of first year's growth.

On July 20 the midribs of twenty-three leaves were inoculated by wounding slightly and spraying with ascospores. In no case was infection secured.

All the inoculations reported in table I were made in bark of varying ages on growth of more than three years. Practically no difference was observed in the susceptibility of old thick bark as compared with younger and thinner bark, except that inoculations were not uniformly as success-

[6] Clinton, G. P. The chestnut bark disease, Diaporthe parasitica Murr. Connecticut Agr. Exp. Sta. Rept. **1907–08**: 879–890. 1909.

[7] Clinton, G. P. Notes on plant diseases of Connecticut. Connecticut Agr. Exp. Sta. Rept. **1909–10**: 716–717. 1911.

[8] Clinton, G. P. Chestnut bark disease. Connecticut Agr. Exp. Sta. Rept. **1912**: 407–413. 1913.

ful in older bark, probably owing largely to the failure to make the wound deep enough to expose unsuberized tissue.

With the exception that young first year twigs are immune in early summer no relation between the seasonal condition of the host and relative susceptibility was observed. Cessation of the growth of the mycelium in the bark during fall and winter as well as negative results of inoculations at this time of year is explained purely on the basis that the temperature is too low for the vegetative activity of the fungus. Thus it happens that this environmental condition which makes growth impossible renders indeterminable under natural conditions what variation there may be in susceptibility during the dormant stage.

No distinctively winter-injured trees were available for inoculations but, from the high percentage of infections obtained from artificial inoculations and the rapid progress which the disease has made in regions in New York under observation, there certainly can be no doubt that the healthiest and most vigorous growing trees are equally susceptible as compared with slow growing, half dead or otherwise injured trees. In cutting some of the trees from the plots used in the inoculation work one tree out of nine showed a distinct ring-shake (pl. XI, fig. B-F). The injury was confined to the rings of wood formed in 1903–04 and was evidently caused by the low temperatures at that time which did much damage to many species of trees. In the subsequent growth of the tree the normal increment of wood accretion had buried this injured zone. No difference could be noted in the general appearance of this tree when compared with those not showing the injured area. The writer knows of no reason by which it may be supposed that this previous injury has had any effect upon the subsequent growth of the tree so that it will be any more susceptible. So that although trees which showed the effects of the winter of 1903–04 were present in the plots it could hardly be considered that in 1912 and 1913 inoculations were being made on trees differing in any way from those not injured in 1903–04. Although the identity of the trees so injured was not determined, no variations in rate of growth and susceptibility were found which would indicate any differences in constitutional vigor due to this injury. As a check on the possibility of any such differences, at least one-half of the inoculations made in the summers of 1912 and 1913 were on coppice trees originating only five years previous (1907). Data from the two groups were practically identical and therefore are not separated in the tables.

The belief that winter-injured trees had furnished a substratum for the development of a parasitic variety of *Endothia radicalis* is now disproved by the recent discovery of the canker fungus in China where it is evidently a native species. That winter-injured trees have played any

part in increasing the epidemic nature of this fungus is becoming more doubtful as evidence other than theoretical is advanced.

In discussing the virulence of *Endothia parasitica* several writers have attributed the extreme susceptibility of the host to the influence of the summer droughts of the past few years. Clinton[9] discusses the probable relations between the winter and drought injury of the chestnut and the appearance and spread of the canker. He says: "Winter injury in 1903–04, aggravated by the droughts, especially that of 1907, we believe to have been important factors in handicapping the trees so that the way was opened for further serious injury by the fungus." He believed the history of the disease up to that time substantiated the statement. Clinton[10] again states, "The increased distribution in the past three years may indicate that the disease has spread to these new localities or it may mean that a more thorough search has revealed its presence and that it has become more prominent because of the four years of drought that have occurred, beginning with 1907."

The writer[11] in 1911 made the following statement: "In September (1911) numerous inoculations were made with all the conditions similar to those which had yielded 100 per cent infection during the summer, except the water and air content of the tree. During the summer there was practically a minimum of water in the trees on account of the drought, but in September a maximum quantity must have been present. In no instance could infection be secured at this time. These chance results together with the fact that suckers and twigs of only one or two years growth are immune" (which is not true) "would point to the possibility that the susceptibility or immunity of the chestnut tree to this wound parasite may vary directly with the increase and decrease of the water content because of the consequent variation in the air content of the tissues." With these observations in mind experiments were conducted during the summer of 1912 to determine the actual effect of drought on the susceptibility of the tree. The experiments were of the following nature: Three plots of trees were laid out representing hill top, south slope and north slope conditions. Each month beginning with May and continuing until September five trees in each of these plots were inoculated and at the same time samples of bark from five trees close to those inoculated were taken and the actual water and air content of the bark determined. For the determination of the water and air content the methods

[9] Clinton, G. P. The chestnut bark disease, Diaporthe parasitica Murr. Connecticut Agr. Exp. Sta. Rept. **1907–708**: 879–890. 1909.

[10] Clinton, G. P. Notes on plant diseases of Connecticut. Connecticut Agr. Exp. Sta. Rept. **1909–10**; 716–717. 1911.

[11] Report read before the American Phytopathological Society at Washington, D. C., 1911. Abstr. in Phytopathology **2**: 99. 1912.

used by Hartig[12] and Münch[13] were followed. The sample of bark was first carefully weighed to centigrams as soon after collecting as possible. Then the volume of the sample was ascertained by the amount of water displaced in an apparatus known as a xylometer. This consisted of a glass jar with a ground rim and lid. The capacity of the jar was determined first by filling with water from a burette. Then, by filling again with the specimen inside, the difference in capacity represented the volume of the specimen. The slight amount of water absorbed was not corrected, since the error represented a constant factor and only comparative results were being sought. The specimens were air dried for eight weeks and then oven dried at a temperature of 110°C. until a constant weight was obtained. The dry weight of the specimens, together with the fresh weight and volume were then used in the formulae given by Hartig (loc. cit.), and the actual amount of liquid water present per 100 grams of bark for each sample was computed. The accompanying table III and figure 2 represent the average water content of the bark of the

TABLE III

*Average water content of bark samples in per cent by weight*

| ASPECT | MAY 2 | JUNE 8 | JULY 11 | AUGUST 1 | SEPTEMBER 10 |
|---|---|---|---|---|---|
| North slope..... | 15.6 | 15.0 | 18.7 | 17.1 | 19.9 |
| South slope..... | 17.5 | 24.8 | 23.9 | 20.4 | 18.8 |
| Hill top..... | 18.7 | 22.2 | 24.2 | 15.8 | 16.4 |

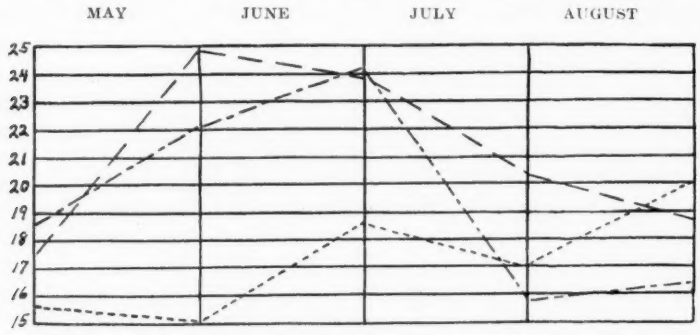



FIG. 2. Graph showing the averaged variation in water content found on the three plots. The figures to the left represent per cent water content by weight.

[12] Hartig, R. Ueber die Vertheilung der organischen Substanz des Wassers und Luftraumes in den Baümen, etc. Untersuchungen aus dem forstbot. Inst. zu München **2**: 1–112. Tab. 1–47, pls. 1–16. 1882.

[13] Münch, Ernst. Untersuchungen über Immunität und Krankheitsempfänglichkeit der Holzpflanzen. Naturw. Zeitschr. f. Forst u. Landwirt. **7**: 54–75. 87–114, 129–160. f. 5. 1909.

trees from which the samples were taken for each month on each of the three plots. Although the summer of 1912 in the region (Napanoch, N. Y.) where this experiment was conducted was not a serious drought year, yet it was very dry for the months of June, July and the first part of August. It will be seen from the table that the water content did not vary to the extent that any difference in absolute or even comparative immunity or susceptibility would be expected according to the results of Münch (loc. cit.) who worked with the wood-rotting fungi. The extremes were 24.8 per cent liquid water in samples taken June 8 from trees

TABLE IV

*Rate of growth of cankers in centimeters*

| HABITAT | STARTED | GROWTH MAY 2 TO JUNE 1 | GROWTH JUNE 1 TO JULY 4 | GROWTH JULY 4 TO AUGUST 2 | GROWTH AUGUST 2 TO SEPTEMBER 17 | GROWTH SEPTEMBER 17 TO OCTOBER 1 |
|---|---|---|---|---|---|---|
| North slope | May | 1.4 | 1.1 | 3.3 | 2.3 | 1.8 |
| | June | | 2.7 | 1.9 | 2.4 | ? |
| South slope | May | 1.3 | 1.7 | 3.3 | 2.3 | 1.7 |
| | June | | 2.5 | 1.8 | ? | ? |
| Hill top | May | 1.4 | 2.4 | 3.0 | ? | ? |
| | June | | 2.5 | 1. | ? | ? |

TABLE V

*Result of the inoculations made at the same time the water content was determined*

| DATE | NORTH SLOPE | | SOUTH SLOPE | | HILL TOP | |
|---|---|---|---|---|---|---|
| | Number made | Number infections | Number made | Number infections | Number made | Number infections |
| May 2 | 20 | 20 | 20 | 20 | 20 | 20 |
| June 2 | 43 | 40 | 53 | 50 | 47 | 47 |
| July 4 | 32 | 19 | 40 | 40 | 40 | 35 |
| August 1 | 41 | 15? | 48 | 18? | 35 | 27 |
| September 10 | 50 | 10? | 50 | 10? | 50 | 6? |

Majority of the inoculations which did not produce infection were made with the hypodermic needle.

on the south slope to 15 per cent on the same date for samples from trees on the north slope. On the other plots the extremes were not so great.

In tables IV and V are found the results of inoculations made on trees in these three plots at the time of taking the bark samples. The figures represent careful monthly observations and measurements of growth of cankers from over five hundred inoculations of different types. The results of these do not show that any great variation in susceptibility or

rate of growth of the mycelium in the bark occurred (for the weather conditions of the summer of 1912) and, furthermore, the variations found may be explained by the other environmental factors which were not considered, mainly temperature. The exceptional negative results obtained from inoculations made with spores in September are explainable by the fact that they were made with the hypodermic needle. As a large majority of the inoculations made in August and September were of this type, the lower percentage of infections secured is due wholly to the technique and not to any difference in susceptibility, as the other types of inoculations gave as good results as in the previous months. It does not appear therefore from the foregoing experiment that such variation in the physiology of the tree which results from drought conditions alters to any great degree either the susceptibility of the chestnut tree or the rate of progress of the mycelium in the bark.

That the chestnut tree has been subject to epidemic troubles before is well attested by many writers. Clinton[14] quotes from many references and summarizes the subject inferring that the fungus now known to cause the canker may plausibly have been the cause of these previous ravages. It seems however from (1) the results of numerous inoculations made by many writers, (2) the continued spread of the fungus year after year and (3) the finding of the fungus native in China, that there remains no doubt that the epidemic now present in this country is the first due to this pathogen, and that the American chestnut without regard to injurious effects of environmental conditions is perfectly susceptible in the healthiest condition. That there has been an inherent decline in the vigor of the species seems to the writer to be only a provisional ground for accounting for its susceptibility to this pathogen, which no longer is needed. Unless then such a decline can be substantiated by future results of research, it may be disregarded along with the winter- and drought-injury theories.

## PATHOGENESIS

It has long been recognized that the canker fungus when growing parasitically produces the so-called mycelial-fans which have come to be the criterion for establishing the identity of the fungus in the field. Growing saprophytically or when only slight growth, if any, is made while the tree is in the dormant condition, these fans are not produced. Examination under the microscope shows that the fans represent fan-like plates of parallel hyphae which spread out between the bast fibers by destroying the parenchymatous tissue between them. The host cells just in advance of the edges of the fan are disintegrated and form a distinct gelatinous

[14] Clinton, G. P. Chestnut bark disease. Connecticut Agr. Exp. Sta. Rept. **1912**: 407–413. 1913.

band which can be seen with the naked eye. Fans are formed in the same way also when the mycelium grows between the phloem and xylem replacing the cambium. The young fans are white but the older ones become tan-colored.

### *Rate of growth of cankers*

During the summer of 1912 over fifteen hundred artificial inoculations were made at Napanoch, New York. New inoculations were made each month and careful records taken of the rate of growth of the resulting cankers, the measurements being made every twenty-eight days. The measurements were made from the external evidence of the spread of the mycelium in smooth bark of young trees. At first, longitudinal growth as well as the growth around the limb was recorded, but since the longitudinal growth proved to be extremely variable these measurements were discontinued. The records were seriously broken in mid-summer through the substitution of injection inoculations for those made in slits, and these did not lend themselves to continuous records because of the lower percentage of infections secured and the slow growth the first two months after inoculation. Table VI shows the results obtained as to the rate of growth of cankers for different months during the summer. One hundred cankers with the most complete records showed an average rate of growth of 1.88 cm. per four week period for the months of June, July, August and September, the maximum growth being 2.1 cm. for August and about 1.75 cm. for the other months. For the period from October 1 until November 15, the rate of growth decreased to 1.56 cm. for a four week period. All cankers made a slight growth from November 15 until April 15 when the next measurements were taken.

TABLE VI

*Average rate of growth of cankers during summer months*

| DATE MADE | NUMBER OF INOCULATIONS | GROWTH BY FOUR WEEK PERIODS IN CM. | | | | |
|---|---|---|---|---|---|---|
| | | May | June | July | August | September |
| May 2 | 60 | 1.35 | 1.75 | 3.0 | 2.4 | 2.3 |
| June 1 | 48 | | 2.5 | 1.72 | 3.0 | |
| June 11 | 16 | | | 2.0 | 1.9 | |
| July 3 | 41 | | | 1.75 | | |
| July 11 | 105 | | | 2.0 | 1.7 | 1.7 |
| August 1 | 11 | | | | 2.1 | |
| Average | | 1.35 | 2.08 | 2.1 | 2.1 | 1.9 |

In considering the results of these records, it is apparent that the rate

of growth is less than one would be led to believe from the previous literature concerning the rapidity with which limbs are girdled. It is certain that practically all the growth is confined to the months from April to October, and, taking the average growth of 1.88 cm. for each four week period, a total growth of about 12 cm. for the season could be expected. Therefore, a limb two inches in diameter would not be girdled in one season even if infection occurred at the earliest possible time in the spring. Also at this rate of growth, it would require a period of about eight years for a single canker to girdle the trunk of a tree a foot in diameter.

### *Pycnidia*

Pycnidia develop abundantly in from three to four weeks after inoculation where wounds are made. They consist externally of small blisters in the bark with ruptured apices from which the conidia are pushed in long yellow tendrils. On removing the layers of cork cells forming the blister, the pycnidium is found to be a ball of apparently loosely aggregated white or slightly buff-colored hyphae. Such young pycnidia have a single globose cavity lined with slender conidiophores which cut off the small pycnospores from their tips. Later, when a stroma begins to be formed around the pycnidium, the cavity assumes a more labyrinthiform character. A very indefinite opening is formed at the apex which serves as an ostiole for the escape of the pycnospores which are, under dry conditions, pushed out in "horns."

The writer[15] called attention to another form of pycnidium which si often found on the wood underneath the loosened bark and on the cut ends of stumps and logs. These occur singly without a stroma and are superficial. They are pear-shaped and have a short beak when growing under moist conditions, such as under the loosened bark of an old canker Where exposed to dryer conditions they are more flattened and lie close to the surface. They are red in color with the exception of the beak-like ostioles which are orange or yellow. They measure about 0.25 mm. in diameter and are slightly taller. Short spore-horns have been seen protruding from the distinct ostioles. Examination of the pycnospores, pure cultures from the same and successful inoculations from tbe pure cultures were made by the writer in 1911 proving the genetic connection of this form with *E. parasitica*. Clinton[16] makes the following statement concerning these superficial pycnidia: "They have an appearance to the eye quite different from those on the bark, and for this reason Saccardo formed a distinct genus Endothiella for them." This statement is errone-

[15] Rankin, W. H. The chestnut tree canker disease. Phytopathology **2**:99. 1912.

[16] Clinton, G. P. Chestnut bark disease. Connecticut Agr. Exp. Sta. Rept. **1912**: 407–413. 1913.

ous, as reference to Saccardo's description[17] will show that he erected the genus Endothiella to include both of the pycnidial forms of *Endothia gyrosa*. The pycnidial stage of *Endothia parasitica* is almost, if not identical with that of *E. gyrosa*. In both species there are the subcortical and superficial types. In erecting the genus Endothiella, Saccardo made a correct place for the classifying of these pycnidial forms. They cannot belong to the genus Cytospora where many writers have erroneously placed them.

Observations were recorded in 1912 on cankers resulting from inoculations made each month, as to the time of appearance of pycnidia and the spore-horns. The small blisters on the bark were often noticed in three weeks after the inoculations were made. The conidial spore-horns appeared within one month after inoculations were made in which mycelium was used. Where ascospores and conidia were used, a slightly longer time was required. This period of approximately one month for the production of conidia was quite constant.

On cankers of more than one year's growth the pycnidia are formed on the advancing portion where the mycelium is invading new tissues.

Inoculations made late in the fall, at the time when but little growth of the mycelium was taking place, failed to produce conidial strings during the winter. The blisters on the bark are produced and from these in the spring the horns are pushed out in abundance.

In 1911 certain cankers were observed carefully. During June, spore-horns were produced from the pycnidia then mature, but during the extremely dry weather, from the first part of July until the last of August, the pycnidia which formed produced only blisters in the smooth bark. The cork layers were not ruptured until frequent rains began the first of September at which time spore-horns were produced in abundance. Sections of the pycnidia made during this dormant period showed them to be mature and full of pycnospores.

It seems that the dehiscence depends entirely upon the continual cutting off of pycnospores which, when the cavity becomes filled, are pushed out through the loosely-woven hyphae at the apex in tendrils, if exterior conditions are dry. In cultures, the spores are exuded in drops instead of horns because of the moisture present. In dry weather the horns of pycnospores grow to be very long and are much curled and twisted. They are very hard and brittle.

### *Pycnospores*

The yellow tendrils are composed entirely of the small hyaline pycnospores held together by a binding substance. When placed in water, the

[17] See Ann. Myc. **4**: 272. 1906.

tendril first swells and turns white, then the binding substance dissolves and the spores float away free from each other. They average about 3.7 by 1.5$\mu$ in size, are oblong or cylindrical with rounded ends, or slightly oval, usually straight but sometimes slightly curved. The spore wall is smooth and colorless.

During periods when no precipitation occurs for some time, and especially when the position of the canker is such that it is protected by grass or in a more or less shaded position, the spore-horns develop very luxuriantly.

The earlier publications contain many assertions as to the dissemination of the pycnospores, the majority of which are merely plausible and not based on any experimental data. The question is one which is more or less difficult to answer. Nevertheless there are many characteristics of these spores and their dehiscence which preclude many methods of dissemination which have been suggested.

As pointed out above, spore horns are produced from the portion of the canker newly formed each year during the spring and summer months. In dry weather the spore-horns are very hard and brittle and of a character such as would make it difficult to believe they could be detached readily by wind currents and, even if they were detached, that they could be carried any great distance owing to their weight. Animate carriers which would come in contact with the horns when dry might transfer the horns to other trees and effect inoculation but it seems that the chances are small for such dissemination.

The characters of these spores, however, when more or less moisture is present, make it possible that they may be disseminated in a great variety of ways. The first and most important of all is by water itself. The spore-horns become separated into the individual pycnospores, on contact with the smallest drop of water. Rain, when thoroughly wetting the cankers, causes all the spore-horns present to "dissolve" and millions of pycnospores are thus transferred to other parts of the same tree or other trees, through the dropping or splashing of the rainwater. It is conceivable that every infection court on a given tree which has one or more cankers in the upper portion may be expected to become infected by pycnospores carried in this way.

The writer performed experiments in which a number of wounds were made below cankers with pycnospore-horns in the fall of 1912. Other check wounds were made on other trees near by. In the spring of 1913, those made below cankers showed practically 100 per cent infection, while the checks developed only a few cankers. The fact that these cankers were due to pycnospores alone could not be proved. In the inoculation experiments reported above a high percentage of infection

was obtained where these conditions were duplicated artificially by spraying conidia suspended in water into slight wounds in the bark.

The majority of the pycnospores must ultimately reach the base of the tree where a saprophytic life may be initiated. The assertion by Metcalf and Collins[18] that these spores "may be washed down into the dust and so blown about with the dust" seems unlikely, since it would be exceptional to have any dust at the immediate base of a chestnut tree, especially in the woods where the fungus is disseminated as rapidly if not possibly more so than out in the open. While still in suspension in water it is plausible that animate agents may transfer the spores from one place to another. When the water which contains the pycnospores is evaporated, the spores are sticky and become firmly attached to whatever object they may come in contact with so that further transfer without being wet again would be improbable. The transfer of the moistened spores by birds, insects, squirrels, etc., is entirely likely when once they come into contact with them, which they must do in badly affected regions. In the opinion of the writer, the pycnospores carried in this way furnish means of only a small percentage of the rapid inoculation of previously healthy trees.

The pycnospores will not germinate in tap or rain water. In decoctions of bark of chestnut and other trees and on certain agar media, they germinate readily. Previous to germination the pycnospores swell until they are from three to four times their normal measurements. Then they germinate, usually, by two germ tubes, one from each end.

Spore-horns collected on May 20, 1912, and stored in dry stoppered bottles were placed in hanging drops of chestnut bark decoction, April 10, 1914. No germination was secured.

Spore-horns collected in June, 1913, and kept under similar conditions were placed in bark decoction at the same time. Good germination was secured in forty-eight hours.

### *Stromata*

On cankers resulting from inoculations made in May, June and July stromata were produced about September 10. Before this time the cankers had been producing spore-horns from the pycnidia which are largely covered by the cork layers of the bark. By active growth a pseudoparenchymatous stroma is developed around this pycnidium which pushes through the cork layer and spreads out cushion-shaped on the bark. While developing, the stromata are first orange and later change, when mature, to reddish and chocolate-brown. In moist situations the stromata

[18] Metcalf, H., and Collins, J. F. The control of the chestnut bark disease. U. S. Dept. Agr., Farmers' Bul. 467: 1–24. 1911.

develop much larger than in drier places. On smooth bark they are generally isolated and slightly elongated at right angles to the axis of the limb. In rough bark the stromata develop in the crevices and are united into long rows. Mature stromata have on the surface numerous projecting papillae, the length of which varies greatly. At times under dry conditions they scarcely project above the surface of the stroma, while in moist and shaded places they may extend out to a length of 2 mm. or more. A single stroma may show from a few to twenty-five or thirty of these papillae. They are the same dark brown color as the rest of the stroma when mature.

Stromata were not produced on any of the cankers until about the second week of September, at which time they appeared on cankers produced by inoculations of June 1 and July 4 as quickly as on those made on May 1. Stromata appeared by October 1 on cankers produced by inoculations made on August 1, only eight and one-half weeks from the time of inoculation, while on those made on May 1, eighteen weeks were required.

*Perithecia*

At the tip of each papilla is a black spot, which is the opening of a long black neck that forms a canal from the perithecium buried in the stroma. A cross section of a stroma shows the globose sac-like perithecia at the base and connected with one of the papillae by the long black canal. The perithecium wall is a leaden-gray color while the wall of the neck is shining black. When mature the cavity of the perithecium, which measures about 0.5 mm. in diameter, is filled with asci each containing eight two-celled spores. The asci are formed at the base of the perithecium and are then detached as others form. The cavity is thus filled with detached asci. Occasional long, slender, simple paraphyses are found. Lining the border of the neck are numerous projecting hyphal branches which are the periphyses (pl. XI, fig. A).

The asci are broadly clavate and average 52 by 9$\mu$. Each ascus contains eight spores, which are arranged in no regular fashion, sometimes uniseriately but more often partially biseriately. The wall of the ascus is more or less thickened at the tip. In some cases the thickened walls leave only a very narrow line-like canal up to the tip, while in others the thickening is only slight and the end of the lumen is truncated. The nature of the two refractive circles at the tip of the lumen was undetermined. Where the walls were very much thickened these circles appeared as imbedded deeply, sometimes closer to the tip than to the lumen, but careful staining showed a narrow canal reaching from the lumen to the circles. Sometimes this canal develops v-shaped branches, the circles

being at the two ends. In this case the substance of the wall of the tip extended down between the circles. The fact that the circles usually stain more deeply than the epiplasm and often even differently seems to prove them to be different chemically. It seems also from their staining action that they represent a solid ring-like structure the function of which was not determined.

As mentioned above, the perithecial stromata develop abundantly in the autumn around the old pycnidia. Examination of stromata formed on cankers produced from inoculations made at different times from May 1 to August 1 showed uniformly mature perithecia and ascospores by the middle of November.

The writer[19] in June, 1911, discovered that the ascospores are forcibly ejected from the perithecium. Upon thoroughly wetting the mature stromata, the papillae swell and the ostioles appear under the hand-lens as partially gelatinized. The asci which usually fill the perithecium are pushed up through the neck due largely to the swelling of the asci themselves. If free water is present on the surface of the stromata even in so slight a quantity as a drop at the ostiole, the spores are forcibly ejected into the air by the rupture of the ascus at the surface of the film of water. If the film is evaporated quickly the asci continue to crowd out of the ostiole but then they only accumulate in a ball. Not all the perithecia of a single stroma mature and eject spores at the same time. As to how long a single perithecium will continue to eject spores was not determined but several small pieces of bark were observed under laboratory conditions to eject ascospores continuously for over a week. In the spring of 1913, after several weeks of frequent precipitation, it was found that spores were not ejected in any quantity from the majority of the perithecial material collected in the field. Whether this was due entirely to the lack of sufficient asci in the then mature perithecia or due to temperature relations was not determined but it seemed on microscopic examination that the former was responsible, for the perithecia contained asci only in the lower one-fourth. About a month later an abundance of spores were ejected from material gathered in the same locality. The record of over a week's continuous shooting was furnished by this material. The change may have been due to the formation of more asci in the old perithecia or to the maturing of new perithecia in the same stromata. The latter occurs commonly and accounts for the fact that while some eject spores others in the stroma do not. Whether more asci are produced in the perithecia which have ejected their original contents was not determined.

The dehiscence may be observed with a hand lens or the 4 and 16 mm.

[19] Rankin, W. H. The chestnut tree canker disease. Phytopathology **2**:99. 1912.

objectives. In either case the only visible phenomenon is the sudden and regular breaking of the film of water over the ostiole. This gives the impression for the moment of a point of light. That the entire content of each ascus is ejected at once is demonstrated by catching the spores on a dry slide. Examination shows that the majority of the spores caught are aggregated in groups of eight or that two or three neighboring groups complete the eight. These seem to be stuck together with a mucilaginous material which probably is the epiplasm present in the mature asci. Upon addition of water or when the spores are ejected upon wet slides the spores are immediately separated. This would seem to prove that the breaking of the ascus must occur above the surface of the film of water or they would become separated and not remain in groups of eight.[20]

Under field conditions mature perithecia have been found at all times of year, and spores are ejected in large numbers whenever the stromata are wet during the spring, summer and autumn. Pure cultures of the fungus are easily made by inverting sterile media in petri dishes above moistened stromata. Abundant material for making inoculations was obtained also by supporting glass object slides over moistened bark and then washing the spores thus caught into atomizers. Such a suspension of spores is opalescent. It was determined that the spores were ejected vertically upward to a height of about six millimeters, and that under ordinary conditions the contents of an ascus was ejected every second or at least every two seconds. At the rate of eight spores every two seconds a single perithecium would eject 14,400 spores per hour. When it is considered that there are from fifteen to thirty perithecia in each stroma and that an abundance of stromata occurs upon a small area of diseased bark, the number of ascospores ejected during rain periods is enormous. The writer has examined stromata in the field during rain periods the last three summers and has found spore ejection constant under natural conditions.

As mentioned above, the asci lying free in the top of the perithecium are pushed up through the long canal to the ostiole when abundant free water is present on the surface of the bark. It is presumed that the size of the perithecium does not change as it is tightly imbedded in the mixed stroma composed of fungous hyphae and the collenchyma of the bark. Therefore, all the increase in size of each ascus and its contents upon the imbibing of free water is exerted in extruding from the perithecium an equal volume of asci. The asci taken from perithecia in active cankers have very thin walls which are collapsed about the spores. Upon the addition of free water the ascus swells quickly to about three times its former volume. This swelling is due mainly to the imbibition of water

[20] A summary of these results was published in Phytopathology **3**: 73. 1913.

by the epiplasm and the ascus wall which appears much thicker, especially at the tip. The asci in this condition emerge to the outside and lie free in the film of water. Then, as stated above, certain asci coming to the surface of the film forcibly eject their contents into the air.

Asci studied in hanging drops show that there is a gelatinization of the ascus wall. The wall at the tip soon becomes so thick that the upper one-third of the lumen is entirely or nearly closed. The wall of the remaining lower portion does not swell in proportion and the ascospores are forced to occupy the lower end of the ascus. The spores themselves soon begin to swell due both to the increase in size of lumina and walls. The maximum swelling results in an increase in volume of the spores alone of more than four times their original volume. This results in the distention of the lower part of the ascus so that the ascus becomes flask-shaped. If observed continuously for twenty-four hours the ascus wall is seen to gradually become thinner and less distinct, until finally it is indistinguishable except for the very upper tip which originally was very much thickened. If the material is not agitated the spores lie imbedded in the matrix of epiplasm and begin to germinate. But if the drop is transferred to a slide and covered by a cover-glass, the spores become separated and no trace is to be found of the walls of the asci. Likewise in mounts made of the gelatinous material from the surface of a stroma which had been dehiscing spores, no trace of the ruptured asci can be found. With these data the following conclusions are drawn as to the process of the actual ejection of spores from the asci. The imbibition of water results in the asci being forced out through the ostiole, where if a film of water is present the wall begins to gelatinize. Undoubtedly as more and more asci are pushed out some arise in the water to the surface and may even project above the surface, due in part to the inertia of being squeezed out between the periphyses at the ostiole or to the crowding of the asci themselves. It is then to be supposed that the increased pressure on the ascus wall due to the swelling of the contents, at a time when the wall is gelatinizing, will result in the sudden rupture of the wall when the ascus arises to or above the surface of water. As the lower portion of the ascus wall is the thinner and gelatinizes and dissolves first, freeing the spores, and as no change suggesting pore or other type of apical dehiscence was found, it is presumed that what proves to be a successful method of dehiscence is due merely to a combination of physical factors and not to any structural arrangements.

### *Ascospores*

The ascospores are ellipsoidal with rounded ends and divided in the middle into two cells by true septa. There is a definite sinus at the sep-

tum. An average of one hundred measurements gave 8.8 by 4.4$\mu$. The walls of the ascospores are much thicker than those of the conidia.

In considering the ways in which ascospores are disseminated, the fact that they are forcibly ejected into the air is of vital consideration. By examining the water at the ostiole while spores are being ejected, it is found that many unruptured asci as well as liberated ascospores are present, so that these may be washed down the tree and disseminated in the same manner as the conidia. However, as the majority are ejected into the air the larger factor in their dissemination is at once apparent, that of their being carried by air currents. Buller[21] has demonstrated by numerous experiments how extremely slight air currents serve to carry spores of certain of the Fungi. Also he has shown that the spores are sufficiently buoyant to be carried for a considerable distance before they fall. When these facts which were demonstrated in closed vessels are borne in mind, it is reasonable to suppose that these ascospores would

TABLE VII

*Results of exposing sterile media near trees with dehiscing perithecia*

| NUMBER OF PLATES | DISTANCE FROM TREES | TIME EXPOSED | RESULTS |
|---|---|---|---|
| 2 | 8 feet | 5 min. | 1 colony |
| 2 | 15 feet | 5 min. | 0 |
| 2 | 3 in. | 5 min. | 2 colonies |
| 2 | 5 feet | 5 min. | 1 colony |
| 10 | 50 feet | 10 min. | 0 |

be blown long distances by even a gentle breeze. Owing to their sticky nature, however, they would be arrested on contact with any object so that in the woods a large percentage of them would be filtered out of the air. That those, however, which find lodgment on chestnut trees may be further transferred by rain water, just as conidia are, is to be expected. The writer believes that the majority of primary infections of healthy trees is brought about by wind blown ascospores. While the carrying of ascospores in any of the means suggested in discussing conidia is also possible, the agents here concerned cannot possibly equal in number the percentage of successful inoculations that millions of buoyant ascospores can which are wind borne. To demonstrate that they can be isolated from the air in the neighborhood of dehiscing stromata, the results of a single experiment are sufficient.

On July 20, 1912, eighteen petri dishes containing sterile media were exposed, three hours after a heavy rain of four hours duration (table VII).

[21] Buller, A. H. R. Researches on fungi, pp. 1–287. 1909.

Conditions were unfavorable in this experiment for the best results, since the stromata had become somewhat dried, and therefore fewer spores were being ejected. However, as the table shows, colonies were obtained in plates held for five minutes within eight feet of the trees.

The ascospores will germinate in tap or rain water. They require no rest period after the perithecium becomes mature. Ascospores developed on cankers produced in the summer of 1912 germinated in November. They swell somewhat, and average about twice the normal measurements. A germ tube is put out from the tip of each cell and these may grow to a length equalling three or four times that of the spore in from ten to fifteen hours after they are placed in water at ordinary summer temperature. Several hours later another tube from the side of each cell may be put out in addition.

Perithecial material, composed of short strips of thin bark collected about the first of June, 1913, ejected spores the third week of January, 1914, giving a record of eight months. This material had been kept perfectly dry under herbarium conditions. Material collected at times previous to this failed to eject spores.

Ascospores from material collected June 11, 1911, September, 1912, November, 1912, and June, 1913, were placed in tap water to germinate the last week of December, 1913. A high percentage of the spores from the material of the last three dates germinated. Those of June 11, 1911, however, practically all produced primary and secondary buds but they did not send out germ tubes. This material like that above had been kept under dry herbarium conditions since it was collected. From these results it is proved that ascospores kept dry in the perithecia will germinate after thirty-one months.

Ascospores ejected upon glass slides and kept dry do not retain their viability as long. The writer has secured germination of ascospores kept in this manner for a period of thirteen weeks.

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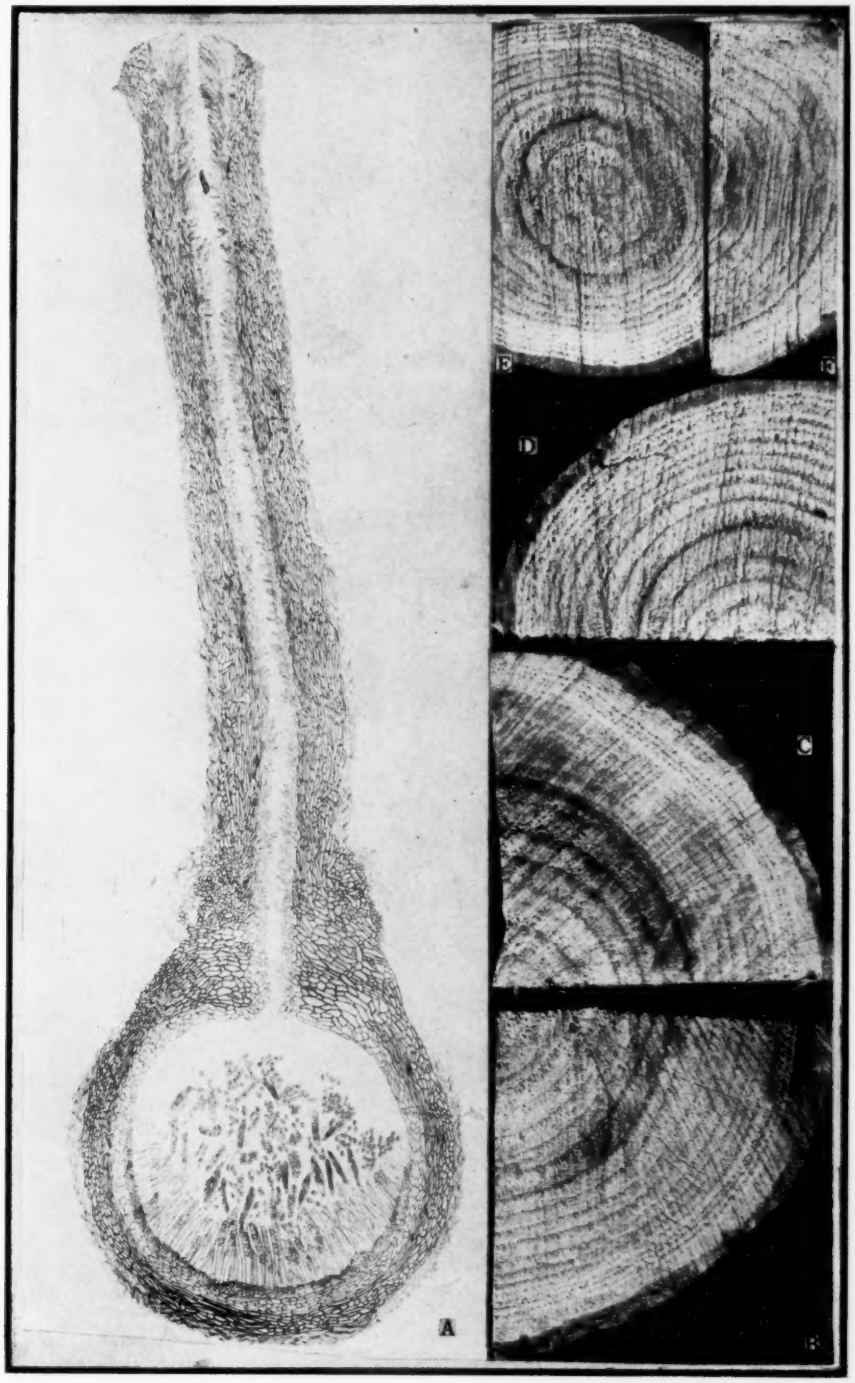
EXPLANATION OF PLATE XI

FIG. A. Showing structure of the perithecium. Camera lucida, magnified 155 diameters.

FIGS. B and C. Annual ring formed 1903-04 showing as a dark zone. Section from base of tree.

FIGS. D. and E. Sections from the same tree ten and twenty feet, respectively, above the base of tree.

FIG. F. Showing slight darkening of the 1910 layer.




RANKIN: ENDOTHIA CANKER

# A NEW POMACEOUS RUST OF ECONOMIC IMPORTANCE,[1] GYMNOSPORANGIUM BLASDALEANUM

H. S. Jackson

With Plates XII and XIII and One Figure in the Text

A rust on the cultivated pear has been known in Oregon for a number of years. It seems to have first attracted attention in 1907 when specimens were received from a number of different localities. Since that time it has been sent in occasionally by correspondents and was particularly abundant during the spring and summer of 1913. The same rust has also been found to occur on quince and apple as well as other hosts of less economic importance. This fungus is particularly interesting since it proves to be a true Aecidium and in this respect differs from all other aecial rusts occurring upon the cultivated pear, apple, or quince. All others are of the Roestelia type and all are known to be connected with various species of Gymnosporangium.

An examination of the literature shows that there are only three species of true Aecidium recognized by mycologists which occur on Pomaceous hosts. These are as follows:

*Aecidium Blasdaleanum* Dietel & Holway (4)

This species was originally described from northern California on *Crataegus rivularis* Nutt. and *Amelanchier alnifolia* Nutt., and has since been reported by Kern (8, 9) as occurring on the latter host and *Amelanchier florida* Lindl. from Oregon and on *Amelanchier pallida* Greene from California and *Crataegus Douglasii* Lindl. from both northern California and Oregon.

[1] Contributions from the Department of Botany and Plant Pathology, Oregon Agricultural College, No. 3.

As read before the Atlanta meeting of the American Phytopathological Society, December, 1913, with the addition of the results of cultural experiments made in the spring of 1914. See abstract, Phytopathology **4**: 41. 1914. The mention of Sorbus hybrida L. in the abstract is herein corrected to Sorbus Spuria Pers.

The writer wishes to acknowledge his indebtedness to Dr. F. D. Kern, who verified the determination of a series of specimens of the rust under discussion and to Mr. Geo. H. Godfrey who assisted in the details of the cultural experiments.

*Aecidium Sorbi* Arth. (1)

Originally recorded on *Sorbus occidentalis* (S. Wats.) Greene from British Columbia, and since reported by Kern (8, 9) as occurring upon the above host from Washington, on *Malus rivularis* (Doug.) Roem. from British Columbia and on *Sorbus Scopulina* Greene from Alaska.

*Aecidium Pourthiaeae* Sydow. (12)

Recorded on *Pourthiaea villosa* (Thunb.) Dec. from Japan.

Arthur (2) has shown in 1908 in connection with cultural experiments that, following inoculation with teliosporic material of *Gymnosporangium Libocedri* (P. Henn.) Kern. from *Heyderia decurrens* (Torr.) K. Koch. (*Libocedrus decurrens* Torr.) from Eugene, Oregon, infection occurred on Crataegus with abundant production of pycnidia and aecia. He records failure on *Amelanchier canadensis*. These results were confirmed in 1911 by the same author (3) who secured infection on *Amelanchier vulgaris*, *Crataegus tomentosa*, *C. cerronis*. Pycnia developed on all three hosts with aecia only on the first. A failure to infect *Sorbus aucuparia* is recorded.

In these experiments the aecia secured, as stated by Arthur (2), "agree perfectly with *Aecidium Blasdaleanum* being wholly lacking in Roestelial characters." It is to be noted that no culture work has been conducted by using any of the native hosts on which *Aecidium Blasdaleanum* naturally occurs. This Aecidium, however, is so distinctive that there would seem to be no doubt of the correctness of the conclusion that *Aecidium Blasdaleanum* and *Gymnosporangium Libocedri* are genetically connected. Kern (8, 9) later uses the combination *Gymnosporangium Blasdaleanum* (D. & H). Kern. He suggests also that *Aecidium Pourthiaeae* may be a synonym of this species since it is morphologically very similar. No telial form, however, has as yet been found in Japan with which this might be genetically connected.

It should be noted that *Aecidium Sorbi* is also a Pacific coast form, but all the collections which have been made up to the present time have been made in localities considerably north of the range of *Heyderia decurrens* and of *Aecidium Blasdaleanum*. *Aecidium Sorbi* is distinctly different from *Aecidium Blasdaleanum*, having much larger aeciospores and peridial cells. Kern (7) has made the interesting prediction that *Aecidium Sorbi* will be found to be genetically connected with an undiscovered telial form and therefore (8, 9) uses the combination *Gymnosporangium Sorbi* (Arth.) Kern and even suggests the possibility of relationship between a cedar rust, *Uredo Nootkatensis* Trelease (11), on *Chamaecyparis Nootkatensis* (Lamb) Spach. which occurs in the same range. This is an espe-

cially interesting prediction since up to the present time no known species of Gymnosporangium has a uredinial stage in its life history.

The first record of the occurrence of Aecidium on pear in Oregon which the writer has been able to obtain is found in the correspondence files of the Oregon Agricultural Experiment Station which show that specimens of the rust, presumably the one under discussion, were sent in from Roseburg in Douglas County, Medford in Jackson County, Lebanon in Linn County, and Creswell in Lane County, in May, 1907. Unfortunately these specimens were not preserved, but from conversation with Professor A. B. Cordley who carried on the correspondence, it seems quite certain that the specimens referred to are of the same species as the one the writer has since observed. The same disease was sent in again, once during the spring of 1911 from Star, Lane County, and frequently from various localities during May and June, 1912.

The writer first saw this Aecidium on pear in the field in the spring of 1912 at Riddle, Douglas County, Oregon. A serious outbreak was reported by a correspondent and the writer visited the locality to investigate the possibility of the relation of this form with *Aecidium Blasdaleanum* and the local cedar rust. It was interesting to note that in this case fully 50 per cent of the fruit of one pear tree, the only one occurring in the particular locality, was affected by this disease. Not more than 200 feet away was a grove of *Heyderia decurrens* with *Gymnosporangium Blasdaleanum* occurring abundantly upon the leaves. In the immediate vicinity *Aecidium Blasdaleanum* was found abundantly upon the leaves and fruit of *Crataegus Douglasii*.

Since the above mentioned observations were made, the writer has examined specimens of the disease on fruit of *Pyrus communis* from all the counties mentioned above, except Jackson County. In all cases it was found to occur within a reasonable distance of Heyderia affected with *Gymnosporangium Blasdaleanum*. Usually the affected cedars were quite close. At Halsey in Linn County, however, where the disease was abundant in 1913, affected cedars were not found in the immediate vicinity but were known to occur in the hills at a distance of about seven or eight miles with a level country intervening. Reports indicate that the disease was not uncommon in the Willamette valley from Eugene to Albany and eastward to the foot hills of the Cascade Mountains. Yet the cedar occurs naturally in the valley only for a short distance north of Eugene along the Willamette River. Cedars are occasionally planted but these have, as a rule, not been found affected. All of the region mentioned is, however, within eight or ten miles of the foothills where *Heyderia decurrens* occurs naturally.

On the pear, infection seems to occur about the time the trees are

in blossom, and it is not uncommon to find pycnia on the base of petals as well as on sepals and ovaries. Mature aecia were found sometime later on the young developing fruits. Under these conditions there was always considerable deformation. The disease also occurs upon the leaves, but not as commonly as on the fruit. Affected fruits usually drop early so that in July it is difficult to find the disease present.

As noted above this disease on the pear is liable to prove of considerable economic importance. In several cases the writer has observed large percentages of fruit infected. Mr. C. E. Stewart, Fruit Inspector, of Cottage Grove, in a letter dated May 9, 1913, stated "Almost every fruit on some trees is now affected. . . . . The Bartletts do not seem to be as badly affected as some of the other varieties."

No experiments for the control of the trouble were conducted. It was observed, however, that trees sprayed with 1–30 lime-sulphur solution for the pear scab just before blossoming were less seriously affected than unsprayed trees. Should a single application at this time fail, on future experience, to satisfactorily control the trouble an additional spraying, one week or ten days previous to the first scab spray, would doubtless aid in reducing infection.

In July, 1912, specimens of quince fruit affected with a similar Aecidium were sent in from Eugene, Oregon, with a statement that half of the fruit on the trees showed infection. These specimens showed remarkable distortion. The writer personally visited this locality and found the disease not only on the fruit, affecting fully 50 per cent, but also very abundantly upon the leaves where the spots were usually small with only a few aecia in each spot. Occasionally the twigs were attacked causing enlargements and distortion. The disease on quince was again observed in the season of 1913 at Halsey, Oregon, and was sent in by correspondents from Creswell and Eugene.

Since this disease is found so commonly upon the pear and quince it occurred to the writer that other hosts might be affected. A search was made, therefore, for the aecial form on the apple, but this was never personally collected by the writer. A specimen on the leaf of a Jonathan apple was, however, sent in by Mr. Stewart of Cottage Grove, who has taken a great deal of interest in aiding the writer in making observations on this trouble. A specimen on the fruit of Winter Banana apple was sent by Mr. J. A. Holt of Eugene. Other observers have reported the occurrence on apple. Specimens were also collected on two cultivated species of Sorbus at Cottage Grove, first by Mr. C. E. Stewart and later by the writer. On these hosts the fruit only was affected. These hosts were provisionally determined as *Sorbus sambucifolia* Roem. and *S. spuria* Pers. In the vicinity of Cottage Grove the disease was also found abun-

dant on fruit and foliage of *Malus rivularis*. This host is known to hybridize commonly with the cultivated apple. Such hybrids were commonly found affected both on fruit and foliage. In addition to the above mentioned host, the disease was found on the ovaries of a flowering crab, the exact species of which was not determined, but it is apparently the

SMALL WITCHES BROOM ON INCENSE CEDAR CAUSED BY GYMNOSPORANGIUM BLASDALEANUM

plant distributed by western nurserymen under the name of *Malus floribundus* Siebold. The disease was also found at Cottage Grove on the foliage of what was apparently the same species not yet of sufficient age to blossom.

The writer has carefully examined specimens of the Aecidium on all

of these hosts and all agree in every respect with descriptions of *Aecidium Blasdaleanum*. It is important also to note that wherever this form has been found on cultivated Pomaceous hosts, *Aecidium Blasdaleanum* is locally abundant on *Crataegus Douglasii* and *Amelanchier alnifolia*. This Aecidium has never been collected either on native or cultivated hosts, except within the range of *Heyderia decurrens* affected with *Gymnosporangium Blasdaleanum*.

In the spring of 1913 an attempt was made in the laboratory to inoculate pear, apple, and quince with teliosporic material of *Gymnosporangium Blasdaleanum*, but without success. The writer now recognizes that the conditions were not ideal for inoculation and no importance is to be placed on these negative results.

Early in February, 1914, potted trees of *Pyrus communis* L. and *Cydonia vulgaris* L. were removed to the greenhouse, and when well started into growth an attempt at infection with teliosporic material from *Heyderia decurrens* was made. The first experiment was set up February 20 but the teliospores were immature and no infection resulted.

On March 21 another set of experiments was started, using the following method. A platform was built so that a large bell jar could be set over the top of each tree which it was desired to infect. Branches of cedar collected by the writer in Eugene containing abundant teliospores were placed under the bell jars and so arranged that the sporidia would fall on the unfolding leaves. The foliage of the cedars as well as the tree to be infected was thoroughly moistened at the beginning of the experiment. Bell jars were kept over the trees for three successive days and nights, being removed every day for a few moments and the inside of the jars moistened and the foliage sprinkled. During the experiment the trees were kept shaded from the direct rays of the sun. One quince and two pear trees of the Bartlett variety were used in this experiment. All became abundantly infected, showing the first signs of infection about March 30. Mature pycnia appeared about April 10 and the fully matured aecia on April 18.

The experiment was repeated in the greenhouse, beginning March 30, using teliosporic material sent from Cottage Grove. Two pear trees and one apple were used. Both pears became abundantly infected, resulting in mature aecia about April 30. Infection started abundantly on the apple but in no case did the aecia mature. Small black spots finally resulted.

The rust in question has never been found naturally in Benton County, in which Corvallis is located, although the writer has made repeated and very careful search for the telial stage on such cultivated trees of cedars as occur in this locality, and for the aecial stage on various pomaceous

hosts, both native and cultivated. On this account it was considered that field infection experiments would be fully as conclusive as those in the greenhouse. Consequently on March 21 infected cedar branches from Eugene were placed in tin cans containing water and hung among the branches of three pear and three quince trees on the college grounds. Fortunately excellent weather conditions for infection prevailed, for some days after the experiment was set up, and in all cases abundant infections resulted on the quinces, and scattered infections on the pears. Mature aecia were collected May 12.

On the same date, cedar branches from the same source as those used in the experiments outlined above were hung in a tree of *Amelanchier alnifolia* which was in blossom, and also in a tree of *Crataegus Douglasii*. On March 30 branches were hung in another tree of *Crataegus Douglasii* and in *Malus rivularis*. These native trees were visited on May 12 and abundant infection with mature aecia was found on *Amelanchier alnifolia* with particularly abundant infection upon the young developing fruits. Quite abundant infections were found upon the foliage of both Crataegus trees and scattered infections were found upon the foliage of *Malus rivularis*.

In connection with all of the experiments where infection was obtained on trees out of doors, whether cultivated or native, it was found that infection occurred only in the immediate vicinity of the cedar branches. In the case of the quince trees infection occurred only on the side of the tree where the branches were hung. The abundance of infection in all cases decreased with the distance from the infecting material. Other trees in which cedar branches were not placed, but which occurred in the immediate vicinity of those used in the experiment, were carefully examined and in no case was infection found.

In all experiments both in the greenhouse and in the field, the aecia developed were typical of *Aecidium Blasdaleanum*.

Based upon the results of these cultural experiments and upon the morphological characters of the aecia the writer believes that the form studied on all the hosts mentioned above is the aecial form of *Gymnosporangium Blasdaleanum* and the following hosts should, in the opinion of the writer, be recorded as new for this species: *Cydonia vulgaris* L., *Malus Malus* (L.) Britton, *Malus rivularis* (Doug.) Roem., *Malus rivularis* × *Malus Malus* (natural hybrid), *Malus floribundus* Siebold, *Pyrus communis* L., *Sorbus spuria* Pers., *Sorbus sambucifolia* Roem. (?).[2]

The telia of *Gymnosporangium Blasdaleanum* are considered by all systematists as developing from an annual mycelium. While making

[2] Since the preparation of this manuscript aecial material on Cydonia japonica Pers. was collected by G. H. Godfrey at Eugene, Oregon. (Plate XII, fig. 6.)

observations on affected cedar trees in connection with these studies, however, "witches' brooms" were found to be very abundant. In fact they were always observed to occur on old trees affected with *Gymnosporangium Blasdaleanum*. An examination was made of many of these and the leaves were always found to be abundantly covered with the characteristic telia of *Gymnosporangium Blasdaleanum*. An examination of the tissues showed mycelium always present. In the older parts of the "witches' broom" the mycelium is most abundant in the pith. "Witches' brooms" were found in all stages of development and always covered with telia. They may reach a large size, sometimes two feet in diameter. The perennial nature of this species is being further investigated.[3]

In certain of the above described infection experiments cedar branches from the "witches' brooms" were used in comparison with infected branches which did not show any hypertrophy. In all cases infection occurred equally well and no difference could be observed in the resulting aecia, indicating that the rust found on the "witches' broom" is the same species as that found on the normal branches of the cedar.

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[3] Since the preparation of this manuscript my attention has been called to similar observations on the witches broom caused by the rust under discussion, made by Dr. E. P. Meinecke, p. 35 of "Forest Tree Diseases common in California and Nevada." U. S. Dept. Agr., Forest Service (Issued February 14, 1914).

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## EXPLANATION OF PLATES

### Plate XII

Fig. 1. Aecia on fruit of cultivated quince.
Fig. 2. Aecia on twig of quince.
Fig. 3. Aecia on leaf of quince.
Fig. 4. Aecia on leaf of Malus floribundus.
Fig. 5. Aecia distorting fruit of cultivated pear.
Fig. 6. Aecia on fruit of Cydonia japonica Pers.
Fig. 7. Aecia on leaves of pear.

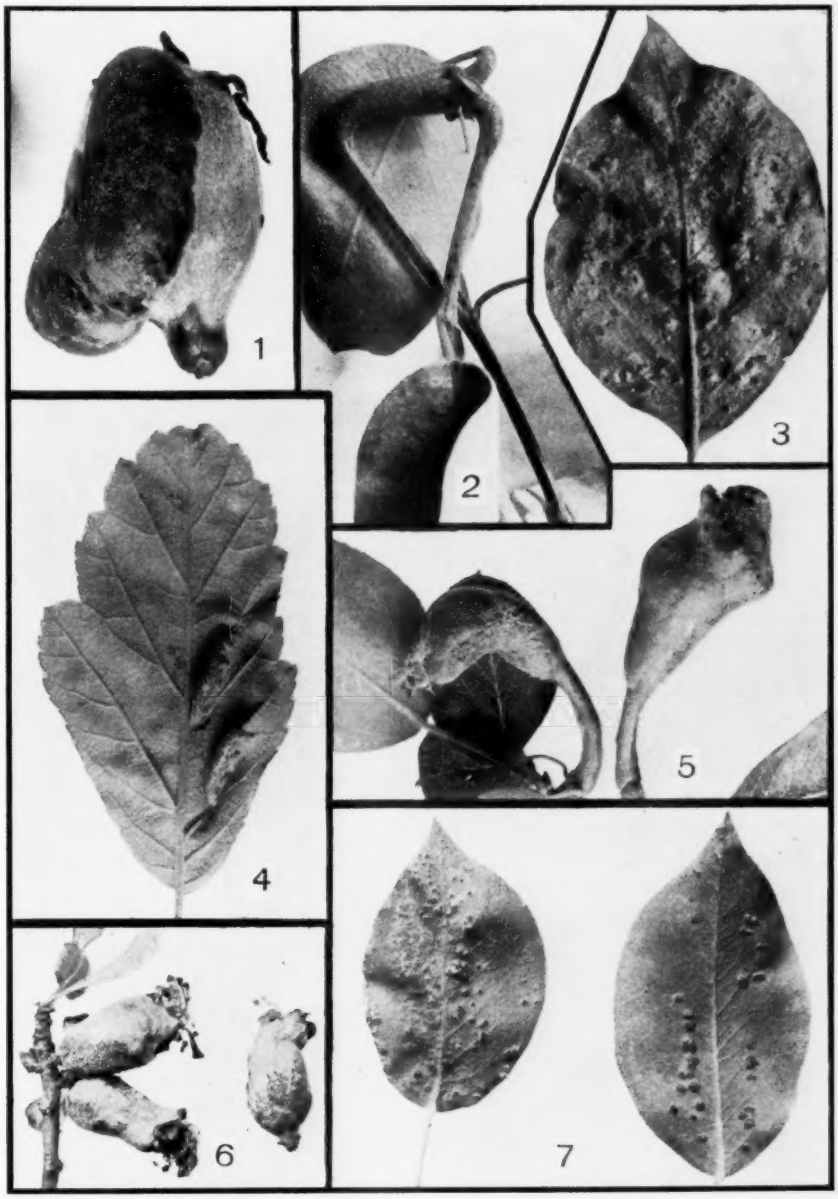


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Plate XIII

Fig. 1. Aecia on leaf of Johnathan apple.

Fig. 2. Aecia on fruit of Malus rivularis. Note persistence of calyx on affected specimens.

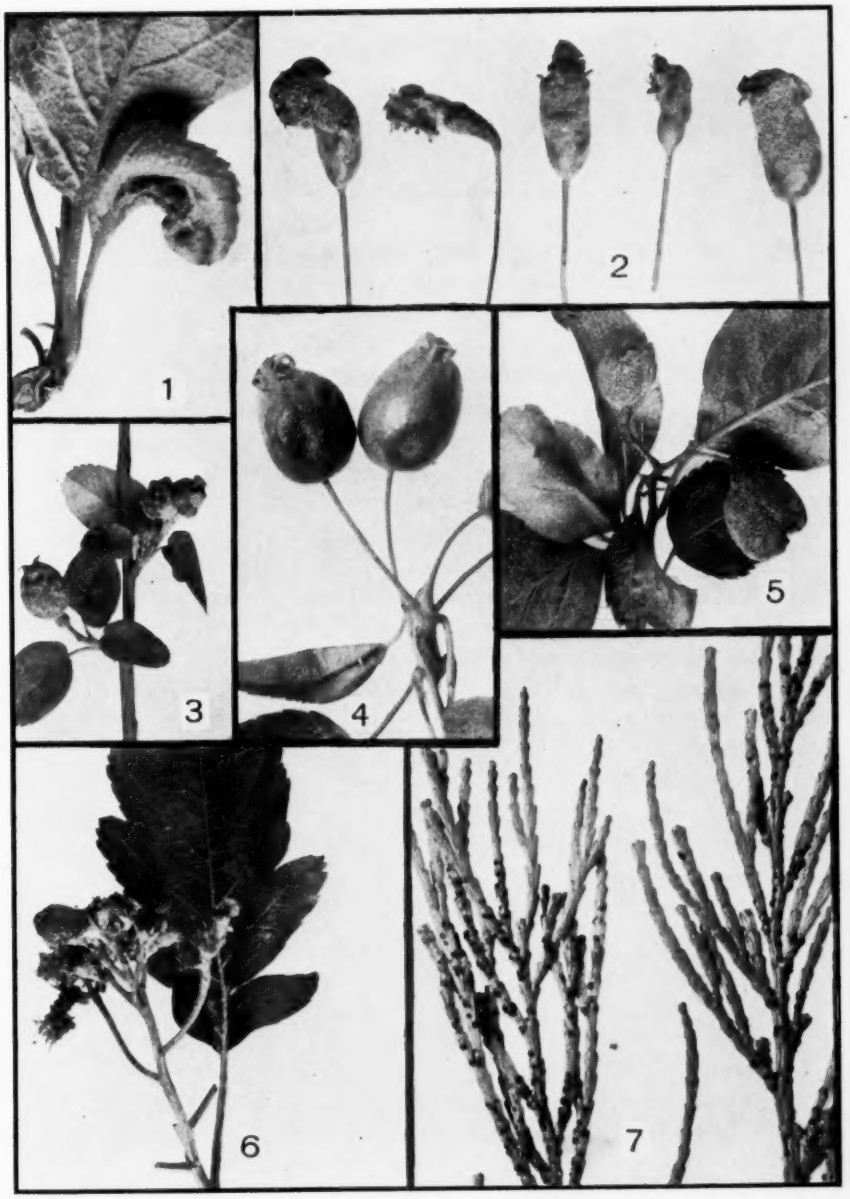
Fig. 3. Aecia on fruit of Amelanchier alnifolia.

Fig. 4. Aecia on fruit of hybrid between cultivated apple and Malus rivularis.

Fig. 5. Aecia on fruit and foliage of Crataegus Douglasii.

Fig. 6. Aecia on fruit of Sorbus spuria.

Fig. 7. Telia on leaves of Heyderia decurrens.




JACKSON: A NEW RUST

# NOTES ON WOOD DESTROYING FUNGI WHICH GROW ON BOTH CONIFEROUS AND DECIDUOUS TREES. I

JAMES R. WEIR

In the course of investigations of the fungi inhabiting forest trees in the northwestern states, the writer has found many new and unusual hosts for certain fungus species heretofore supposed to be strictly confined to coniferous or deciduous trees. The subject, therefore, seems worthy of the following short account, especially since a number of species supposed to be confined to deciduous trees are found to be serious sapwood destroyers in the coniferous forests of the northwestern regions.

As a rule, a line can be drawn between the fungi ordinarily growing on the wood of coniferous or deciduous trees, but, when the usual host is absent, another of an entirely different nature is frequently selected. The number of tree fungi, semiparasitic or saprophytic, confined to one class of wood only, are really very few and those always associated with particular genera are rarer still. In the former class may be mentioned such species as *Polyporus schweinitzii* Fr. and *Fomes laricis* (Jacq.) Murr., never found, as far as the writer is aware on anything but coniferous hosts. *Echinodontium tinctorium* E. & E. is practically confined to two genera, Tsuga and Abies. *Polyporus borealis* (Wahlenb.) Fr., apparently never occurs on Pinus but always on Picea, Tsuga or Abies. In this region the fungus is almost entirely confined to Picea. *Polyporus alboluteus* E. & E. is principally associated with Picea. The number of wood-destroying species showing a pronounced predilection for coniferous or deciduous hosts, or for particular genera, has been growing constantly smaller with the more detailed surveys in all parts of the Northwest.

A fact that must not be overlooked in the correct determination of a species growing under a great range of conditions and on all manner of substrata, is the great variation induced in the appearance of the sporophore. Failure to observe these influences closely and in the minutest detail, has led to the creation of many so-called "new species," the names of which sadly burden the literature and work a hardship on practical investigators.

In a recent paper Romell[1] gives a number of host variations for European conditions. The species cited are common to both Europe and America and likewise exhibit considerable variation in the selection of hosts in this

[1] Some fungi growing both on coniferous and deciduous trees. Mycologia **1**, no. 6: 265. 1909.

country. In the preparation of the present paper, the large representative collections of forest fungi from the Northwest, and from other sections of the United States and Europe, preserved for study in the Laboratory of Forest Pathology at Missoula, Montana, have been thoroughly consulted. All observations and species here given are supported by material collected by the writer.

*Hydnum coralloides* Scop. In the forests of Idaho and Washington we have a large branching Hydnum that is the cause of a very serious heart-rot in *Abies grandis*. It is also occasionally collected on *Picea engelmanni*. Whether this ungus is *H. coralloides* or *H. caput-ursi* Fr., is difficult to determine. It seems to be an intermediate form between the two. Perhaps it is closer to *H. coralloides*. *H. caput-ursi* is usually a compact species, more on the order of *H. erinaceus* Bull., and like it has large spines. These are the characters frequently noticed for the fungus in question. On the other hand, it is sometimes more branched and diffused with small spines. If the plant should prove to be a new species, then it has no place in this paper, as it is in all probability closely associated with its coniferous host. If *H. coralloides*, it is cosmopolitan, and is abundant on the wood of deciduous trees, especially on Populus.

*Stereum hirsutum* Willd., which is frequent in the West on various hard woods, occasionally occurs on conifers, usually branches of *Abies grandis* and *Tsuga heterophylla*.

*Stereum purpureum* Pers., which usually grows on Populus, Salix, and Betula, has been collected on moist branches of *Larix occidentalis*.

*Hymenochaete tabacina* Sw. is common on both conifers and broad-leaf trees in the Northwest.

*Trametes pini* (Brot.) Fr. This fungus apparently has never been reported as growing on deciduous wood. However, the writer has recently made a collection on *Betula occidentalis* in the Kaniksu national forest, Idaho. In deep narrow valleys containing a mixed stand of conifers, practically every tree of which is infected with this fungus, it is not surprising that an occasional infection of deciduous species occurs.

A small brown Trametes, which could easily be confused with *Trametes pini* or again with *Trametes abietis* Karst, is quite common in the West on species of Crataegus. *T. abietis* has setae, so also has *T. pini*. The fungus on Crataegus has very few setae and its rot is quite different from that of either of these species. Mr. C. G. Lloyd, to whom specimens were submitted, is of the opinion that the fungus is *Trametes piceinus* Pk. In Professor Peck's description of this species no setae are mentioned. The fungus is, moreover, interesting since it occurs on the trunk and branches of living trees which eventually succumb to the attack. It is apparently confined to the genus Crataegus in the West.

*Trametes suaveolens* L. This is a common fungus throughout the West on Populus and Salix species, and is frequently parasitic on the latter, killing the host. A sterile abnormal growth of *T. suaveolens* has been observed on *Abies grandis*. This specimen could very well be referred to the so-called genus Ptychogaster. In the damp forest regions of Idaho many abnormal polyporoids occur, partly spore-bearing, partly conidial; they can usually be referred to the proper species, showing beyond doubt that the present conception of the "genus" Ptychogaster, as being the conidial condition of various polypores, is well founded.

*Polyporus giganteus* (Pers.) described by Murrill as growing "about old stumps and trunks of deciduous trees," is associated with conifers in the forests of Idaho, more often in connection with old decayed roots of Douglas fir. Romell[2] reports it in association with *Pinus silvestris*.

*Polyporus frondosus* Fr.[3] and *P. umbellatus* (Pers.) are also frequently found throughout the coniferous forests of Idaho and Washington, but never in connection with any kind of sound wood.

*Polyporus berkeleyi* Fr. has recently been collected by the writer[4] in the Kaniksu national forest of Idaho, particularly associated with the roots of *Larix occidentalis* and has been found to cause a decay of the wood in roots wounded through fires and other causes. In the East this species is almost invariably found growing at the base of oaks. In a recent publication Long[5] ascribes a very serious butt rot in the white oak forests of Arkansas to this fungus.

*Polyporus picipes* Fr. This black velvet stemmed form of *P. varius* of Europe is common in Idaho and Washington on Populus. It has also been collected on *Picea engelmanni* and *Abies grandis*.

*Polyporus chioneus* Fr. This species, usually found on wood of deciduous trees is quite common on conifers in the Northwest, principally on *Thuya plicata*. It has also been collected on *Pinus monticola* and *Tsuga heterophylla* and is of some consequence in the destruction of the sapwood of these three hosts when killed by fire.

*Polyporus adustus* Fr., common on *Betula occidentalis* and on species of Populus, is occasionally found on *Larix occidentalis*, *Thuya plicata* and *Pseudotsuga taxifolia*, though very rarely. Romell reports it on spruce in Sweden.

[2] Mycologia **1**, no. 6: 265. 1909.

[3] Polyporus frondosus, according to Murrill (Journal New York Botanical Garden, **7**, no. 82: 223, 1906) attacks the roots of Italian chestnuts and is said to cause considerable damage.

[4] Some observations on Polyporus berkeleyi. Phytopathology **3**: 101–103, pl. 9. 1913.

[5] Three undescribed heart-rots of hardwood trees especially of oak. Journal of Agricultural Research [U. S. Dept. Agric], **1**, no. 2: 109–128. November, 1913.

*Polyporus dichrous* Fr. is common on *Juniperus virginiana* in the vicinity of Washington, D. C., and recently it has been found to be a serious sapwood fungus on various Northwestern conifers. Collected chiefly on *Thuya plicata* it also occurs on *Tsuga heterophylla*, *Pinus monticola* and *Larix occidentalis*. In the East it is chiefly associated with *Quercus alba*. This fungus is also referred to the genus Gloeoporus but is not, according to Lloyd, *Gloeoporus conchoides* Mont., which name while often applied to this plant in American mycology, should be restricted to the thin, pale conchoid tropical form.

*Polyporus sulphureus* Fr. Probably no wood-rotting fungus exhibits such a variety of hosts as this species. In Europe it is common on both coniferous and frondose wood, and in America it attacks practically every native forest tree. Hedgcock[6] gives eighteen broad-leafed trees and twelve coniferous species as hosts for this fungus.

*Polyporus caesius* Fr., which ordinarily occurs in the East on the wood of deciduous trees, is common in Idaho and Washington on *Tsuga heterophylla*, *Larix occidentalis* and *Abies grandis*. Other specimens in our collection are from Liriodendron, Acer and Betula.

*Polyporus benzoinus* (Wahlenb.), common in Idaho and Washington on *Tsuga heterophylla*, *Larix occidentalis* and *Abies grandis*, is undoubtedly the same as *P. resinosus* Schrad., on species of Populus and Acer in the eastern states. Both plants are fragrant, have the same color markings and resinous appearance, and the minute characters are similar.

*Polyporus gilvus* Fr. is thought to be confined to the wood of deciduous trees. The fungus is quite common on *Juniperus virginiana* in the vicinity of Washington, D. C.

*Polystictus aurantiacus* Pk. is considered a very rare fungus in the United States. It is principally associated with *Pseudotsuga taxifolia* and *Tsuga heterophylla* in Idaho and Washington. The writer has recently made a collection from the roots of an overturned maple in the Pend Oreille national forest, Idaho.

*Polystictus versicolor* (L.) Fr. The wide range of deciduous hosts for the species is well known. It has been collected on *Larix occidentalis*, *Pinus monticola* and *Pseudotsuga taxifolia*.

*Polystictus cinnabarinus* (Jacq.) Fr., occurring abundantly in the Northwest on *Betula occidentalis*, *Acer glabrum*, and on species of Populus, Prunus and Salix has recently been collected on *Thuya plicata*.

*Polystictus hirsutus* Fr. One of the most common of all polypores on the wood of deciduous trees has been collected on *Thuya plicata*.

[6] Notes on some diseases of trees in our National forests II. Phytopathology **2**: 76–79. 1912.

*Fomes pinicola* (Swartz.). This species in addition to collections from practically every conifer in the Northwest has been found on *Populus trichocarpa, P. tremuloides, Betula occidentalis, Salix lasiandra* (Selkirk Mountains, British Columbia), *Alnus tenuifolia,* and *Pyrus malus* (varieties). In the collection of the botanical department of the University of Montana is a specimen on a variety of domestic plum. The two last-named collections exhibit a greater variation from the ordinary form, the context and spore characters are the same, but the pileus has a peculiar laccate surface. All the specimens from deciduous hosts resemble somewhat the form known as *Fomes marginatus* Fr., but the margins are yellow rather than red. The writer has collected *F. pinicola* on *Fagus silvatica* and *Quercus pedunculata* in Baden, Germany, where it also occurs on *Alnus glutinosa.* Pennington[7] reports it upon *Fagus ferruginea, Betula lenta* and *Acer saccharum* from the Adirondacks and on the balsam poplars of northern Michigan. The same author, in a recent communication to the writer, reports finding the fungus on *Prunus serotina.*

A most interesting case of an invasion into an orchard of various varieties of fruit trees by forest tree fungi, recently came to the writer's attention in the Priest River Valley, Idaho. The orchard situated in the heart of a coniferous forest had not been particularly well tended. In plowing among the trees, wounds had been made which exposed considerable wood. These wounds had not healed and later became infected with various fungi common in the surrounding forest. The death of a variety of blue plum was directly traced to the activities of *Polystictus hirsutus.* The fungus entered at a wound in the base of the tree and spread upward in the heartwood, later forming small sporophores at the lenticels along the entire main trunk. A wound on the trunk of a living Yellow Transparent apple tree bore a small sporophore of *Fomes pinicola* and an occasional wound was found to be infected with *Stereum hirsutum,* all of which shows that very little is known of the host range of many wood destroying fungi.

*Fomes annosus* Fr., which usually attacks conifers, has been collected on various deciduous trees of the Northwest.

*Fomes leucophaeus* Mont. is frequently found on coniferous hosts in the West[8] and is the cause of considerable damage to fire killed fir in some sections. In our laboratory collection at Missoula are specimens from *Abies grandis, Pseudotsuga taxifolia* and *Tsuga heterophylla* and it is occasionally taken from dead parts of living trees. The plant is more often associated with deciduous hosts, especially species of Populus.

[7] Fomes pinicola Fr. and its hosts. Ninth Annual Report of the Michigan Academy of Science, p. 80–82.

[8] Hedgcock, Notes on some diseases of trees in our national forests II. Phytopathology **2**: 76. April, 1912.

*Lenzites sepiaria* Fr. This species, so common on coniferous wood, is frequently a deciduous tree fungus. In the list of host trees given by Spaulding[9] for this species, the following are noted: *Alnus* sp., *Populus alba*, *P. deltoides*, *P. tremuloides*, *Salix* sp. and *S. discolor*. The fungus has been collected in the Northwest on *Alnus tenuifolia*, *Prunus demissa*, *Betula occidentalis*, *Populus trichocarpa*, *Salix lasiandra*, *Acer glabrum* and *Crataegus Douglasii*. All are very rare hosts, especially the last. This fungus is occasionally found destroying the heartwood of living trees, entering through wounds, and follows close behind the newly-formed sapwood.

*Armillaria mellea* (Vahl.) Quél. is common on both coniferous and broad-leafed trees.

*Pleurotus serotinus* Fr. The common green Pleurotus on species of Alnus and Populus, frequently occurs on *Abies grandis*.

*Pholiota adiposa* Fr. A serious enemy to *Abies grandis*, occurs very rarely on Populus along with *P. destruens* Brond.

*Paxillus atratomentosus* Fr. common on coniferous wood, and more rarely on the wood of broad-leaf species, also occurs frequently on the ground.

The host variations of a number of other species will be communicated at a later date.

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[9] The timber rot, caused by Lenzites sepiaria. Bur. Pl. Ind. Bul. 214: 11.

# THE SOUTHERN BACTERIAL WILT IN NEW JERSEY

MEL. T. COOK

WITH ONE FIGURE IN THE TEXT

During the summer of 1913 there was a great deal of complaint concerning an epidemic which was very destructive to the potato crop of South Jersey. It was described by the growers as a sudden ripening or dying of the crop early in the season before the tubers had reached half their normal size. In some cases the entire crop was destroyed; in others the crop on areas of varying sizes died while the remainder of the crop was in a fairly good condition. In many cases the value of the crop was not equal to the cost of the fertilizers used. The epidemic was most destructive in the immediate vicinity of Mullica Hill and was very severe as far north as Burlington, from which point it decreased rapidly to the northward, there being comparatively little north of Trenton. The growers attributed this epidemic to drouth, hot weather, blight and lightning.

The horizontal lines indicate the most important potato growing area; the perpendicular lines the most important tomato growing area, *1*, Mullica Hill; *2*, Burlington; *3*, Trenton; *4*, Philadelphia.

A careful investigation convinced the writer that this disease was the southern bacterial wilt (*Bacillus solanacearum* Smith). The most observant growers noticed that the plants over considerable areas would wilt during the day, revive at night, wilt again during the second day, but very seldom revive a second time. The plants fall, the stems become amber colored and finally black. It was most severe on the very dry soils, especially those in which a coarse gravel predominated. Irregular areas of dead plants in many fields coincided with the varying grades of soil. A careful examination of these plants and study by means of culture invariably gave the organism, but fungi were comparatively rare. However, black leg (*Bacillus phytophthorus* Appel) and stem blight (*Fusarium oxysporum* Schlecht) were common throughout the potato growing area and were frequently confused with the wilt by the growers.

This disease was first described by Smith[1] in 1896. It is well known as one of the most serious diseases of the southern states but has also been reported from several northern states. In the New Jersey Agricultural Experiment Station Report for 1891, Dr. Byron D. Halsted records a bacterial blight or wilt of the potato which he suspects as being the same as the tomato bacterial wilt which he records in the same volume. In his report for 1894 he reports a "potato bacteriosis" as next to the potato scab in importance and says that "in some parts of the state it is occasionally ruinous to the entire crop." In the same report he says a "bacteriosis of the tomato prevails in many parts of the state and is not, as yet, well understood." Judging from Dr. Halsted's discussions, it appears very probable that these outbreaks were due to *Bacillus solanacearum*. In the 1903 Report he says, "One of the most difficult diseases of the tomato for the trucker to contend with is the bacteriosis, due to a microbe (*Bacillus solanacearum* Sm.), which is often recognized by a sudden wilting of the foliage, that soon changes to a yellowish-green and then to brown."

In this connection it is well to note that the southern part of the state is very little above sea level, that it lies between the Atlantic Ocean on the one side and Delaware Bay and River on the other, and that the climatic conditions are very similar to those of states much farther south. It should also be noted that that part of the potato growing district in which the disease was most severe coincides with a part of the tomato growing districts. Some of our reports by correspondence indicate that the disease also attacked the tomato in 1913, but was not so severe as on the potato. The condition was probably due to rains in the latter part of the season. The severity of the disease on the potato in 1913 was probably due to the very mild winter of 1912–13 followed by the exceptionally dry growing season for the early potato crop.

NEW JERSEY AGRICULTURAL
EXPERIMENT STATIONS

[1] Smith, E. F. A bacterial disease of the tomato, egg plant and Irish potato. U. S. Dept. of Agric., Div. Veg. Phys. and Path. Bul. **12**: 1–26. 1896.

# THE STEM-ROT OF THE SWEET POTATO (IPOMOEA BATATAS)

L. L. Harter and Ethel C. Field

With Plates XIV, XV, and XVI and Two Figures in the Text

## INTRODUCTION

The first published account of the sweet potato (*Ipomoea batatas* Poir.) stem-rot appeared, so far as we know, in 1890 when Halsted (1890) briefly described the disease, but stated that the cause was then unknown. In 1892 he discussed the disease again as "eggplant stem-rot" (Halsted, 1892). He claimed that the stem-rot of the eggplant is caused by the same organism, a Nectria, which he specified as *N. ipomoeae* because it was found by Galloway on the sweet potato two years previously. In 1895 he attributed the ring-rot of the sweet potato to the same organism (Halsted, 1895).

It is uncertain how long the stem-rot has been an important disease of the sweet potato, but probably many years before Halsted began work on it. Old sweet potato growers claim that the disease has been present in the fields of New Jersey to their knowledge for forty-five years. It is barely possible, of course, that they may have confused stem-rot with black-rot, an old and serious disease in that State which is caused by *Sphaeronema fimbriatum* (Ell. & Hals.) Sacc. This is unlikely, however, in view of the fact that these same growers are able to distinguish the two at the present time without any difficulty.

Investigations of the disease were begun in the fall of 1910. The object at that time was merely to conduct field experiments for its control and, with that end in view, a preliminary study was first made of the causal organism. Considerable doubt soon arose, however, as to the real cause of the disease. Inoculation experiments which followed soon led to the belief, and it has later been definitely proven, that *Nectria ipomoeae* Hals. is not the cause of stem-rot, but 2 species of Fusarium unrelated to *Nectria ipomoeae*. This conclusion was reached after the termination of 1654 inoculations, details of which will appear in later pages. Numerous observations on the nature of the disease have been made in the field and experiments are still in progress looking towards the best means for its control.

DISTRIBUTION, PREVALENCE, AND LOSS

The many reports received from collaborators of this office will give some idea of its distribution. It should be stated in connection with these reports, however, that, while some of the collaborators attributed stem-rot to *Nectria ipomoeae*,[1] others doubted the parasitic nature of this organism and reported *Fusarium* sp. as the cause. None of the collaborators, so far as we know, proved the parasitism of the organism they reported. It is probable that they were largely guided by the appearance of the affected plants which is very characteristic and easily distinguished from other sweet potato diseases. It is believed in most, if not all cases that these reports are wholly reliable and indicative of the distribution of the disease.

Reports have been received from California, Illinois, Ohio, New Jersey, Delaware, North and South Carolina, and specimens have been collected by the writers in Maryland, Virginia, Delaware, and New Jersey. Diseased specimens were collected in Alabama by H. M. Connolly which were kindly sent to the writers for study. The parasitism of the organisms isolated from material from the different States and different localities has been proven in most cases by inoculation experiments.

In 1903, A. D. Selby reported stem-rot of the sweet potato from Washington County, Ohio. He again reported its occurrence in 1906, 1907, and 1909 in Washington and Lawrence Counties, and in 1911 in Drake County.

In 1904 R. E. Smith reported the stem-rot as very common and destructive in Merced County, California. It was again reported in 1910 by Rogers from the same county, being quite abundant at Atwater and also from Stanislaus County, though no large losses occurred.

In 1907 the disease was reported by J. T. Barrett to be present in Union County, Illinois.

H. W. Barre reports its occurrence in 1908 and in 1909 in Oconee County, and in 1910 in Oconee and Pickens Counties, South Carolina.

F. L. Stevens reports its occurrence in North Carolina in 1910.

On August 30, 1910, specimens collected at Annapolis, Maryland, were sent in by J. B. S. Norton.

Mel. T. Cook and J. J. Taubenhaus reported the disease from Delaware in 1911, and T. F. Manns in 1912.

The disease is probably more widely distributed and causes greater

[1] There is at the present time considerable difference of opinion regarding the taxonomy of the organism described by Halsted as Nectria ipomoeae, which is identical with Creonectria ipomoeae of Seaver and Hypomyces ipomoeae of Wollenweber. The organism will be referred to throughout this article as Nectria ipomoeae.

loss in New Jersey than in any other state. All fields in the sweet potato section of the state appear to be infected regardless of whether they have ever been planted to this crop or not. In 1911, when sweet potatoes were planted on land which had not borne this crop for forty years or more, 25 to 50 per cent of the plants were diseased the first year and a higher per cent the second year. The prevalence of stem-rot and the loss incurred are not so well known from a personal inspection in other states, although it is commonly found in other states, especially along the Atlantic coast.

While it is difficult to estimate accurately the loss that can be attributed directly or indirectly to this disease, it evidently amounts to many thousand dollars annually. In parts of southern New Jersey where the sweet potato is the principal money crop, the loss in the field varies every year from 10 to 50 per cent, and fields have been seen where 95 per cent of the plants were killed. It is not believed that the organisms cause a storage rot to any great extent, although they enter the roots, causing a brown discoloration of the fibro-vascular bundles, and may be the means of affording an easy entrance to rot-producing organisms.

## SYMPTOMOLOGY

Although Halsted's description of the symptoms of the stem-rot is somewhat brief, there is no doubt but that he was discussing the same disease which is known by that name at the present time. The first indication of the disease in the field is characterized by a slight difference in the color of the foliage from that of healthy plants. The leaves become duller in color, then yellowed between the veins and somewhat puckered, followed by wilting of affected vines (pl. XIV). The youngest or apical leaves are generally the first to show signs of disease, but they usually remain attached to the vine until the plant dies. The older basal leaves, however, especially if the mycelium enters the petiole, drop off. When the attack is severe the young plants often produce a number of short stems at the center of the hill, giving a rosetted appearance. The browning or blackening of the fibro-vascular bundles of diseased vines which can readily be detected by breaking open the epidermis (fig. 1), is very characteristic of the disease. This discoloration of the bundles has been found to extend 3 to 5 feet from the point of infection. In later stages the vines rupture, the surface becomes blackened, and typical spores are formed thereon. The vines may die after forming a few small potatoes. Sprouts an inch or more in length frequently develop from these and are soon invaded by the fungus and killed (pl. XV, fig. 1). The organism passes from the stem down into the bundles of the roots (pl. XV, fig. 2) producing a brown discoloration.

In the hotbeds the symptoms are similar to those in the field. The presence of the parasite can generally be detected in the white part of the stem by a faint purplish tint which is cast by the blackened bundles through the epidermis.

ORGANISMS ISOLATED

A number of species of Fusarium have been isolated from sweet potato stems and from the roots in storage. Which of these were storage rots, true saprophytes, or the cause of stem-rot could only be determined by isolating them whenever found associated with sweet potatoes, and con-

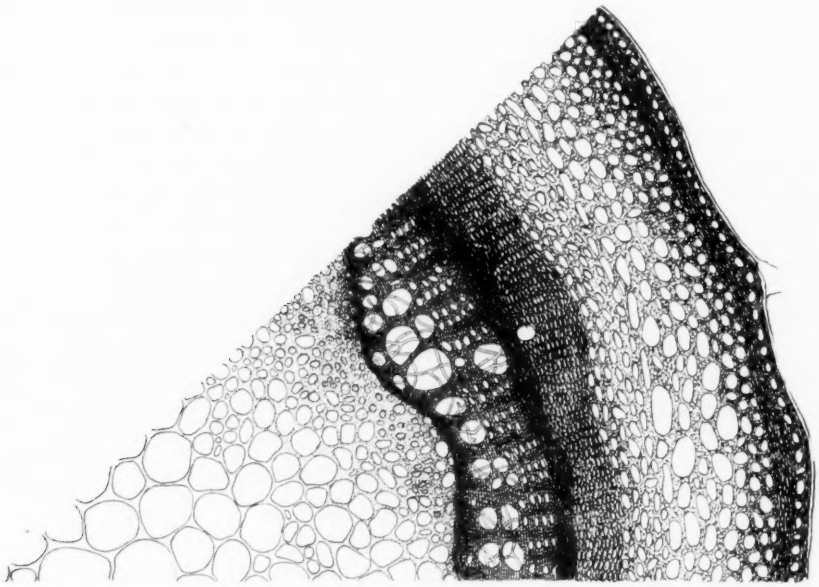

FIG. 1. Camera lucida drawing showing the presence of hyphae in the fibro-vascular bundles of the stem of a diseased sweet potato plant.

ducting inoculation experiments. Sweet potatoes are very susceptible to storage rots and most of the Fusarium species studied have been obtained from storage potatoes. In all, twenty isolations, including eight species, have been made, from which inoculation experiments have been conducted on the living plant. Some of these isolations are recoveries of parasitic forms and were used to inoculate other plants, and some were found to be the same as species designated by another number. All of these and several other species have been inoculated into potatoes kept in storage, the results of which will appear in a later article. The isolations do not include the many recoveries made from inoculated plants for the purpose

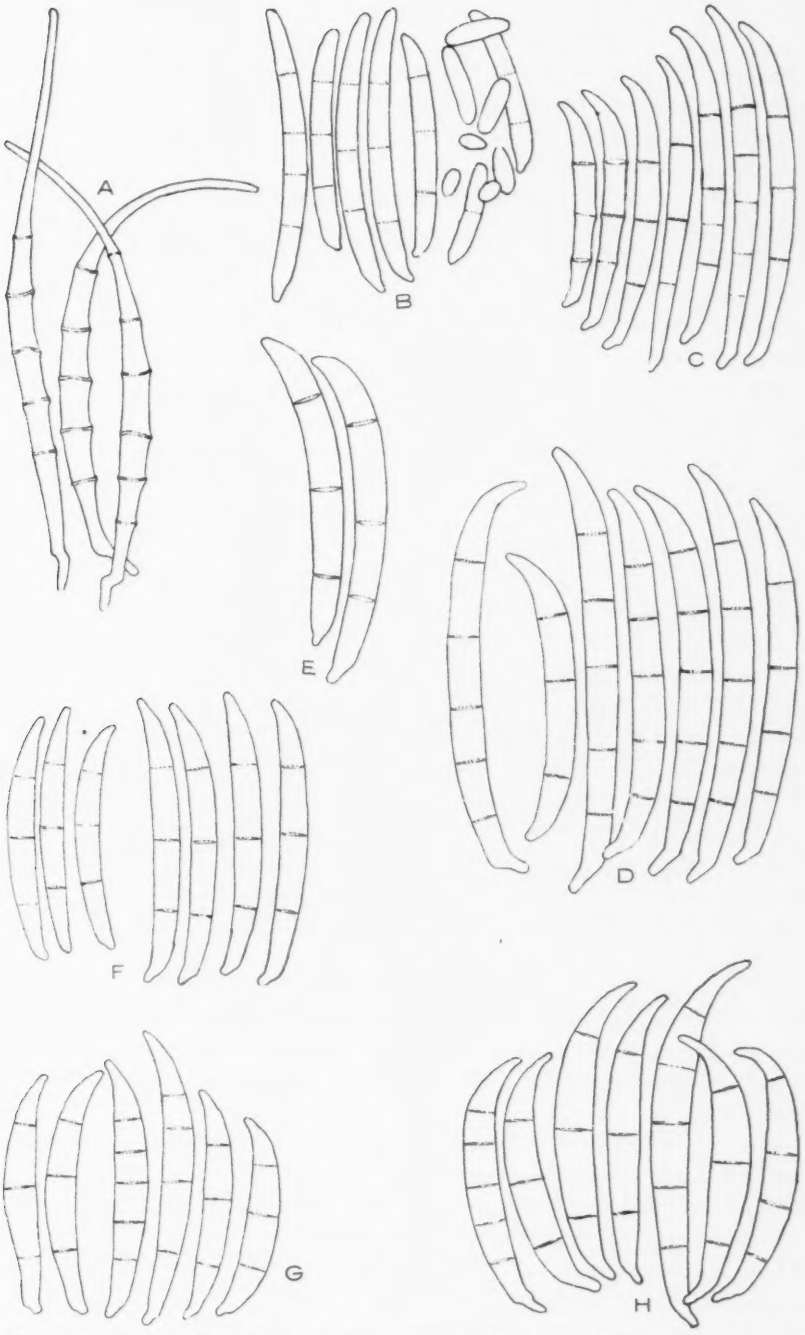


FIG. 2. Conidia of Nectria ipomoeae, Gibberella saubinetii and different species of Fusarium. × 1000.

of comparing them with the original strain and which were carried in cultures only long enough to establish their identity For convenience, each of the organisms will be referred to by a number used in our notes on isolations, inoculations, etc.

The original isolation of each organism was made by planting bits of the diseased tissue in plates of synthetic agar, after thoroughly washing and disinfecting in mercuric chlorid (1–1000) for about forty seconds to one minute. Later transfers were made to beef agar slants. All inoculations, with a few exceptions to be noted later, have been made from cultures descended from a single spore. To obtain cultures from single spores, poured agar plates were made in which several spores were marked by ringing with India ink and later transferred to beef agar tubes. If the tubes from a macroscopic and microscopic examination appeared to be of the same organism, all but one was thrown away and subsequent transfers made only from it, If there was enough difference to indicate that two or more organisms might be present, one of each was kept. The importance of deriving cultures from a single spore can not be overestimated if one takes into consideration the fact that two or three species of Fusarium may be found together in a single decayed potato. All species with which experiments have been made, with a few exceptions, have been determined by H. W. Wollenweber (1914).

No. 1. Identified by Wollenweber as *Fusarium batatatis* Wr. This is a new species and parasitic. The species (fig. 2, *C*) was isolated (January 10, 1912) from the browned fibro-vascular bundles of a sound sweet potato root sent us by Geo. A. Mitchell from Vineland, New Jersey.

No. 2. Identified by Wollenweber as *F. batatatis* Wr. This organism was recovered (February 7, 1912) from the fibro-vascular bundles of the stem of a sweet potato plant inoculated in the greenhouse with cultures from No. 1.

No. 3. Identified by the writers as *Fusarium orthoceras* App. & Wr. This organism (fig 2, *B*) was isolated (April 13, 1912) from a somewhat sunken ring midway between the two ends of a potato collected in a storage house at Vineland, New Jersey (pl. XVI). It is called "ring-rot" by the growers.

No. 4. Identified by Wollenweber as *Nectria ipomoeae* Hals. (See footnote page 280) This organism was isolated (April 4, 1912) from the partially decayed and browned stem end of a sweet potato collected from a storage house at Swedesboro, New Jersey, by Geo. A. Mitchell. This disease is called "end-rot" by the growers. Figure 2, *D*, shows normal conidia of *Nectria ipomoeae*.

No. 5. Identified by Wollenweber as *Fusarium oxysporum* Schlecht. This organism (fig. 2, *F*) was isolated (April 11, 1912) from the browned

root end of sweet potatoes collected from a storage house at Vineland, New Jersey. The disease is commonly known as "end-rot."

No. 6. Identified by Wollenweber as *Fusarium radicicola* Wr. This organism (fig. 2, *E*) was obtained (February 10, 1912) from the browned interior of a partially decayed potato sent by Carleton Weaver, Tulsa, Oklahoma.

No. 7. Identified by Wollenweber as *Fusarium oxysporum* Schlecht. This strain was isolated (February 10, 1912) from a partially decayed sweet potato sent by C. A. Ludwig, a graduate student at Purdue University, La Fayette, Indiana.

No. 8. Identified by Wollenweber as *Fusarium caudatum* Wr. This organism was isolated (January 18, 1912) from the browned interior of a sweet potato sent us by H. W. Barre, Clemson College, South Carolina. In a letter he stated that probably one hundred bushels of potatoes could be found decayed in a similar manner within two miles of the college (fig. 2, *A*).

No. 9. Identified by Wollenweber as *Fusarium oxysporum* Schlecht. This organism was isolated (January 13, 1912) from a sunken decayed spot midway between the ends of a potato collected in a storage house in Philadelphia. The injury was typical of what is generally known as "ring-rot."

No. 10. Identified by the writers as *Nectria ipomoeae* Hals. This strain was obtained (April 29, 1912) from a badly decayed sweet potato from storage at Vineland, New Jersey. Perithecia were formed abundantly on the surface.

No. 12. Identified by Wollenweber as *Fusarium hyperoxysporum* Wr. This is a new species and parasitic (fig. 2, *G*). This species was first isolated (June 20, 1912) from the fibro-vascular bundles of the vine of a plant that had been set out in the field fifteen days previously.

No. 13. Identified by Wollenweber as *Gibberella saubinetii* (Mont.) Sacc. This organism (fig. 2, *H*, conidial stage) was sent to Wollenweber for identification and study by C. A. Ludwig, who kindly permitted the writers to use it for inoculation experiments.

No. 14. Identified by the writers as *Fusarium hyperoxysporum* Wr. This strain was recovered (July 10, 1912) from the fibro-vascular bundles of a plant of *Ipomoea hederacea* Jacq. that had been inoculated with Fusarium No. 12.

No. 15. Identified by the writers as *Fusarium batatatis* Wr. This organism was recovered (September 13, 1912) from *Ipomoeae hederacea* that had been inoculated with Fusarium No. 1.

No. 16. Identified by the writers as *Fusarium hyperoxysporum* Wr. This strain was recovered from the stem of a sweet potato plant that had been inoculated in the axil of the fourth leaf with Fusarium No. 12.

No. 21. Identified by the writers as *Fusarium batatatis* Wr. This strain was isolated (July 10, 1913) from the fibro-vascular bundles of a sweet potato plant sent by W. E. Cox, Federalsburg, Maryland.

No. 23. Identified by the writers as *Fusarium batatatis* Wr. This organism was isolated (September 4, 1913) from the fibro-vascular bundles of the stem of a sweet potato plant collected at Laurel, Delaware.

No. 24. Identified by the writers as *Fusarium batatatis* Wr. The organism was isolated from the fibro-vascular bundles of the stem of a sweet potato plant collected at Blue Anchor, New Jersey, September 10, 1913.

No. 26. Identified by the writers as *Fusarium hyperoxysporum* Wr. This organism was isolated from sweet potato plants sent by H. M. Connolly, from Alabama. Mr. Connolly stated that the disease was rather prevalent on the farm where the specimens were collected, but not widespread throughout that section of the country.

No. 106. Identified by the writers as *Fusarium hyperoxysporum* Wr. This organism was recovered from Irish potatoes that were inoculated in the greenhouse October 9, 1912, with Fusarium No. 12.

## CULTURAL METHODS

As a result of our experience with sweet potato Fusaria, there is no doubt that the condition of the culture at the time of inoculation has a marked influence on the development of the disease. Infection is more likely to take place and the disease progresses much more rapidly when the organisms are cultivated on media where there is a maximum amount of septate conidia and a minimum of hyphae and one-celled conidia. Wollenweber suggested the use of stems or potato cylinders for most species as the best media on which a high culture[2] might be obtained. Agars have proved to be poor media for this purpose as they give a minimum amount of septate spores and a maximum of hyphae and one-celled spores.

The organisms were first isolated by planting bits of diseased tissue in plates of synthetic agar which contained sufficient acid to check the growth of bacteria. The Fusaria would soon outgrow the bacteria if present, making it easy to obtain a pure culture by transfer to tubes of either agar, Irish or sweet potato cylinders or stems. Steamed or autoclaved sweet potato and Melilotus stems were found to be excellent media for the growth of the sweet potato Fusaria. These media were used almost exclusively and only in a few cases have inoculations been made from any others. When transfers were made from plates to sweet potato or Meli-

[2] By a high culture is meant a medium on which a maximum of the septate spores and a minimum of hyphae and one-celled conidia are produced.

lotus stems, typical septate spores were generally formed. If only hyphae were transferred, normal conidia sometimes were not found, but transfers of spores usually gave a high culture. When a high culture was not obtained in this manner, poured plates were made on Irish potato agar, using spores only, and transfers made from the growth which followed to sweet potato or Melilotus stems. This step was repeated several times if necessary to obtain a high culture. On the other hand, if an organism seemed to be degenerating or running out it could often be rejuvenated by several transfers about a week apart to new media. The transfers were preferably made by inoculating a tube of sterile water with spores, care being taken not to wash off the hyphae in the water and then transferring one or two loopfuls to the potato cylinders or stems. The secret of retaining virulent cultures seems to be, so far as our own experience goes, in making rather frequent transfers of spores only and preferably of normal septate conidia. It was found that in general the young or immature stems were better than mature ones for our parasitic forms.

## INOCULATION EXPERIMENTS

In all, 1654 inoculations have been made to determine what species are and are not parasitic. All the organisms studied were found to be saprophytes or only storage rots, except *F. batatatis* and *F. hyperoxysporum*, both of which are parasitic on *Ipomoea batatas* and *Ipomoea hederacea*. While *Fusarium oxysporum* was obtained from some of the stored potatoes inoculated with this organism, we do not consider it a parasite of the sweet potato. This species produced no visible injury to the plants in the field, neither was it confined to those hills inoculated with it, since *F. oxysporum* was isolated from potatoes inoculated with *F. orthoceras*, *F. radicicola*, *F. caudatum*, *Gibberella saubinetii*, *Nectria ipomoeae* and the checks. One hundred and ninety-one sweet potato plants were inoculated with *F. hyperoxysporum*, and 162, or 85 per cent became infected. Three hundred and fourteen plants were inoculated with *F. batatatis* and 195, or 62 per cent became infected. Several other crops, viz.: *Lycopersicon esculentum*, *Solanum tuberosum*, *Capsicum annuum*, *Solanum melongena*, *Trifolium pratense*, *Ipomoea purpurea*, *I. hederacea*, *I. coccinea* and *I. lacunosa* have been inoculated but only *I. hederacea* was infected.

Inoculations of *Solanum melongena* and *Ipomoea batatas* with *Nectria ipomoeae* gave no results that would indicate that this organism caused the stem-rot. Although 15 out of 18 sweet potato plants inoculated with this organism in the greenhouse were diseased, none of these showed any evidence of the typical stem-rot such as is produced by *Fusarium batatatis* and *F. hyperoxysporum*. The symptomology more nearly resembled that

of "damping off" caused by a number of different species of fungi. The plants first showed a blackening of the tissue around the point of inoculation. Unless supported by roots formed above the wound, the plant would later fall over and die. Attempts to isolate the fungus from the fibro-vascular bundles of the green portion of the stem close to the wound have been unsuccessful. Microscopic examinations have also failed to show hyphae in the bundles. On the other hand, the stem-rot organism enters the fibro-vascular bundles and is easily isolated therefrom often several feet from the point of inoculation.

Inoculations of 100 plants in the field with the same organism gave only negative results. It is believed, therefore, that the environment under which the plants were grown had much to do with the results of inoculations with *Nectria ipomoeae*. In the greenhouse there was a relatively high humidity and high temperature. Furthermore, the soil was frequently kept too wet and the plants had little sunshine. From the results it can be concluded that *Nectria ipomoeae* is no more than a weak parasite of sweet potatoes under abnormal conditions and can in no way be regarded as the cause of stem-rot. There was no evidence of injury to other hosts inoculated with *Nectria ipomoeae* except the swollen condition of the stem in some cases at the point where the spores were inserted. The wound soon healed and the plants grew normally thereafter. When cut open the bundles were blackened for a half inch or more each way from the wound. Although *Nectria ipomoeae* was isolated from the bundles of some of the inoculated plants after more than two months, it is not believed that they could be considered diseased, since no apparent harm was done to the plants. In fact, it is quite easy in many cases to recover an organism of this type from the bundles of inoculated plants even though the fungus is not parasitic on the host. Plants of *Solanum melongena* were inoculated with *Fusarium batatatis* and *F. hyperoxysporum*, but no disease was produced thereby.

The source of the cultures from which inoculations have been made has been indicated on pages 286 and 287. Inoculations have been made with the same organisms in the greenhouse and in the field on the Potomac Flats near Washington, D. C.

Every precaution was taken to prevent miscellaneous infections In the greenhouse the plants were grown in pots only. Before using, the soil and pots were steamed for one hour at a temperature of 120°C. at a pressure of 15 pounds. The pots were kept far enough apart so that the vines would not overlap or if the vines grew too long they were cut back. Diseased leaves and vines were carefully disposed of. Our records show that not a single check plant accidentally became infected. Sweet potato stem-rot never before occurred on the Potomac Flats. The plants were grown on made land, the sand and silt having been pumped out of the

river one or two years previous to make the land. Only two plants grown in this field, besides those inoculated by us with *Fusarium bata'atis* and *F. hyperoxysporum* developed stem-rot. *Fusarium batatatis* was isolated from two plants inoculated with *Nectria ipomoeae*. A chance infection of this kind would not be improbable since the plants inoculated with *F. batatatis* and *Nectria ipomoeae* were only three rows apart.

Unless otherwise stated, the inoculations were made at the base of the stem by slightly wounding the surface and inserting spores and hyphae. After inoculation, a little moist dirt was pressed about the stem to prevent the fungus from drying out. Care was always taken not to wound the plant any more than was absolutely necessary to insure the insertion of spores into the fibro-vascular bundles.

When the plants which were inoculated on the Potomac Flats were dug, the stem in each case was split open to make sure that there was or was not an infection. Whether or not there was any evidence of infection the potatoes from each hill were put in a separate cloth bag, carefully labeled and stored. It was suspected in advance, as a result of inoculation experiments in the greenhouse, that most of these organisms were not parasitic on the vine, though it was thought that some of them might be storage rots which had their origin in the growing plant and became injurious only after remaining in storage for some time. The results of this part of the investigation will be found elsewhere in this article.

The following tables show the results of inoculating[3] different hosts with various species of Fusarium and with *Nectria ipomoeae* and *Gibberella saubinetii*.

Because of the large number of plants inoculated, no attempt has been made to recover the organism from every one that was infected. The two parasitic species have been isolated many times, however, from the roots and from other parts of infected plants, and cultures from these strains have been used for inoculating others.

All the plants inoculated in a set did not necessarily show signs of infection at the same time. In fact, some plants would show the characteristic symptoms in about ten days after inoculation while others could not be regarded as unquestionably diseased for four to six weeks. Furthermore, the age of plants appeared to have little or no influence on the degree of susceptibility since the first symptoms of infection appeared on older plants as soon after inoculation as in younger ones. There was one noticeable difference, however. When young plants were inoculated they were generally killed by the organism while frequently old plants recovered in the course of three or four weeks, and grew normally thereafter.

[3] The yellow Jersey variety was used for all inoculation experiments unless otherwise stated.

TABLE I

*Results of inoculating sweet potato plants (Ipomoea batatas) with Fusarium batatatis and hyperoxysporum*

| DATE AND PLACE OF INOCULATION | LENGTH OF EXPERIMENT IN DAYS | ORGANISM NUMBER | NAME OF ORGANISM | METHOD OF INOCULATION | AGE OF PLANTS | NUMBER | | | PERCENTAGE INFECTED |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | INOCULATED | INFECTED | CHECKS* | |
| January 19, 1912, Greenhouse | 99 | 1 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Young | 22 | 17 | 8 | 77.3 |
| April 8, 1912, Greenhouse | 102 | 1 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Young | 5 | 1 | 4 | 20.0 |
| June 20, 1912, Field | 110 | 1 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Young | 100 | 56 | 155 | 56.0 |
| September 20, 1912, Greenhouse | 66 | 1 | Fusarium batatatis† | By inserting spores and hyphae at base of stem | Young | 8 | 0 | 10 | 0.0 |
| February 19, 1912, Greenhouse | 67 | 2 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Young | 15 | 12 | 5 | 80.0 |
| April 8, 1912, Greenhouse | 102 | 2 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Young | 5 | 3 | 4 | 60.0 |
| June 20, 1912, Field | 110 | 2 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Young | 100 | 67 | 155 | 67.0 |
| September 10, 1912, Greenhouse | 48 | 2 | Fusarium batatatis† | By inserting spores and hyphae at base of stem | Fairly young | 7 | 4 | 10 | 57.1 |
| July 22, 1913, Greenhouse | 15 | 21 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Young | 10 | 9 | 10 | 90.0 |
| September 30, 1913, Greenhouse | 17 | 23 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Young | 6 | 6 | 6 | 100.0 |
| October 11, 1913, Greenhouse | 12 | 24 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Young | 6 | 6 | 6 | 100.0 |
| October 1, 1912, Greenhouse | 44 | 15 | Fusarium batatatis | By inserting spores and hyphae at base of stem | Medium young | 10 | 10 | 10 | 100.0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| June 27, 1912, Greenhouse | 36 | 12 | Fusarium hyperoxysporum | By inserting spores and hyphae at base of stem | Young | 12 | 12 | 15 | 100.0 |
| July 1, 1912, Field | 99 | 12 | Fusarium hyperoxysporum | By inserting spores and hyphae at base of stem | Young | 100 | 92 | 155 | 92.0 |
| July 17, 1912, Greenhouse | 111 | 12 | Fusarium hyperoxysporum | By inserting spores and hyphae at base of stem | About 4 weeks in pots | 20 | 18 | 7 | 90.0 |
| July 17, 1912, Greenhouse | 10 | 12 | Fusarium hyperoxysporum | By inserting spores and hyphae at base of stem | Young | 8 | 8 | 4 | 100.0 |
| September 20, 1912, Greenhouse | 146 | 12 | Fusarium hyperoxysporum† | By inserting spores and hyphae at base of stem | Young | 8 | 7 | 10 | 87.5 |
| November 18, 1912, Greenhouse | 88 | 12 | Fusarium hyperoxysporum | By inserting spores and hyphae at base of stem | Young | 4 | 2 | 6 | 50.0 |
| August 28, 1912, Greenhouse | 15 | 14 | Fusarium hyperoxysporum | By inserting spores and hyphae at base of stem | Young | 10 | 10 | 5 | 100.0 |
| December 22, 1913, Greenhouse | 29 | 26 | Fusarium hyperoxysporum | By inserting spores and hyphae at base of stem | Young | 6 | 5 | 6 | 83 0 |
| December 3, 1912, Greenhouse | 73 | 106 | Fusarium hyperoxysporum | By inserting spores and hyphae at base of stem | Young | 5 | 5 | 3 | 100 0 |
| January 24, 1912, Greenhouse | 167 | 1 | Fusarium batatatis | By pouring bouillon cultures on the soil and then setting out plants | Young | 12 | 3 | 6 | 25.0 |

TABLE f—Continued

| DATE AND PLACE OF INOCULATION | LENGTH OF EXPERIMENT IN DAYS | ORGANISM NUMBER | NAME OF ORGANISM | METHOD OF INOCULATION | AGE OF PLANTS | NUMBER | | | PERCENTAGE INFECTED |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | INOCULATED | INFECTED | CHECKS* | |
| October 3, 1912, Greenhouse | 85 | 1 | Fusarium batatatis | By inserting spores and hyphae into the axil of the fourth leaf | Medium young | 8 | 1 | 10 | 12.5 |
| October 3, 1912, Greenhouse | 85 | 12 | Fusarium hyperoxysporum | By inserting spores and hyphae into the axil of the fourth leaf | Medium young | 8 | 2 | | 25.0 |
| September 11, 1912, Greenhouse | 107 | 12 | Fusarium hyperoxysporum | By pouring tubes of sterile water containing the spores about the plants | Young | 10 | 1 | 10 | 10.0 |

* None of the checks were diseased.

† Inoculations made with cultures given the writers by Dr. Wollenweber without revealing the identity of the organism until the final notes were taken. It was found after the termination of the experiments that some of the organisms used were the same as those the writers had given Wollenweber for identification.

It is frequently noticed and often a subject of comment that the vines and potatoes of some of the largest hills in the field have stem-rot. It is believed that such plants usually become infected after they are well started in the field and as a result the organism is unable to do any, or at most, only a little harm. This is a great source of danger to those farmers who select seed from productive hills, believing that productivity is a proof that the potatoes are not diseased. This point, however, will be discussed more fully under dissemination and prophylactic measures.

In several cases when plants were inoculated on the stem some distance from the hill considerable injury resulted. The organism grew in both directions, extending out to the end of the vine and down into the roots and out again into another vine which branched from the main stem. *Fusarium hyperoxysporum*, the organism with which the inoculations were made was recovered from the fibro-vascular bundles both above and below the point of inoculation.

The result of the in culation experiments show that only *Fusarium hyperoxysporum* and *F. batatatis* cause stem-rot of sweet potatoes and *Ipomoea hederacea*.

### STORAGE OF SWEET POTATOES INOCULATED IN THE FIELD

Although *Fusarium batatatis* and *F. hyperoxysporum* were the only species which produced stem-rot, the potatoes from each hill of all inoculated plants were put in a cloth bag and stored at the Arlington Experiment Farm. After about five months in storage, cultures were made from all potatoes that were completely or partially decayed, and from potatoes from hills inoculated with organism Nos. 1, 2 and 12 whenever there was any evidence of browning of the bundles even though no rot had taken place. It frequently happened that only one or two potatoes in a hill rotted or started to rot and cultures were made from these so that when Diaporthe 1 or Penicillium 4, etc., is given, this does not signify that all potatoes from that hill were infected. One hundred plants were inoculated in the field with each species of Fusarium but many of those inoculated with the parasitic forms were killed (No. 1 *F. batatatis* 20; No. 2 *F. batatatis* 28; No. 12 *F. hyperoxysporum* 49) during the summer. In all 415 isolations have been made from wholly or partially decayed stored sweet potatoes. Table IV shows the different organisms isolated and the frequency with which they occur.

TABLE II

*Results of inoculating sweet potato plants (Ipomoea batatas) with Nectria ipomoeae, Gibberella saubinetii, Fusarium oxysporum, F. caudatum, F. radicicola, and F. orthoceras*

| DATE AND PLACE OF INOCULATION | LENGTH OF EXPERIMENT IN DAYS | ORGANISM NUMBER | NAME OF ORGANISM | METHOD OF INOCULATION | AGE OF PLANTS | NUMBER | | | PERCENTAGE INFECTED |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | INOCULATED | INFECTED | CHECKS* | |
| June 17, 1912, Greenhouse | 84 | 10 | Nectria ipomoeae | By pouring cultures about plant† | Young | 9 | 0 | 15 | 0 |
| June 1, 1912, Greenhouse | 87 | 4 | Nectria ipomoeae | By inserting spores and hyphae into base of stem | Young | 6 | 4‡ | 15 | 67 |
| June 20, 1912, Field | 111 | 4 | Nectria ipomoeae | By inserting spores and hyphae into base of stem | Young | 100 | 0 | 155 | 0 |
| May 14, 1912, Greenhouse | 98 | 10 | Nectria ipomoeae | By inserting spores and hyphae into base of stem | Young | 12 | 11‡ | 15 | 91.7 |
| May 27, 1912, Greenhouse | 91 | 5 | Fusarium oxysporum | By inserting spores and hyphae into base of stem | Young | 10 | 0 | 15 | 0 |
| June 1, 1912, Greenhouse | 87 | 5 | Fusarium oxysporum | By inserting spores and hyphae into base of stem | Young | 10 | 0 | 15 | 0 |
| June 21, 1912, Field | 110 | 5 | Fusarium oxysporum | By inserting spores and hyphae into base of stem | Young | 100 | 0 | 155 | 0 |
| May 27, 1912, Greenhouse | 91 | 7 | Fusarium oxysproum | By inserting spores and hyphae into base of stem | Young | 10 | 0 | 15 | 0 |
| September 20, 1912, Greenhouse | 56 | 7 | Fusarium oxysporum§ | By inserting spores and hyphae into base of stem | Young | 8 | 0 | 10 | 0 |
| June 21, 1912, Field | 111 | 7 | Fusarium oxysporum | By inserting spores and hyphae into base of stem | Young | 99 | 0 | 155 | 0 |
| June 21, 1912, Field | 112 | 9 | Fusarium oxysporum | By inserting spores and hyphae into base of stem | Young | 101 | 0 | 155 | 0 |
| June 27, 1912, Greenhouse | 90 | 9 | Fusarium oxysporum | By inserting spores and hyphae into base of stem | Young | 12 | 0 | 15 | 0 |
| September 20, 1912, Greenhouse | 66 | 9 | Fusarium oxysporum§ | By inserting spores and hyphae into base of stem | Young | 8 | 0¶ | 10 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| August 23, 1912, Field | 48 | | Fusarium oxysporum ⁂ | By inserting spores and hyphae into base of stem | Medium young | 10 | 0 | 50 | 0 |
| September 5, 1912, Greenhouse | 49 | | Fusarium oxysporum ⁂ | By inserting spores and hyphae into base of stem | Young | 10 | 0 | 5 | 0 |
| September 10, 1912, Field | 35 | | Fusarium oxysporum§ | By inserting spores and hyphae into base of stem | Fairly young | 7 | 0 | 10 | 0 |
| September 20, 1912, Greenhouse | 56 | | Fusarium oxysporum§ | By inserting spores and hyphae into base of stem | Young | 8 | 0 | 10 | 0 |
| May 27, 1912, Greenhouse | 91 | 8 | Fusarium caudatum | By inserting spores and hyphae into base of stem | Young | 10 | 0 | 15 | 0 |
| June 21, 1912, Field | 111 | 8 | Fusarium caudatum | By inserting spores and hyphae into base of stem | Young | 100 | 0 | 155 | 0 |
| June 1, 1912, Greenhouse | 87 | 6 | Fusarium radicicola | By inserting spores and hyphae into base of stem | Young | 12 | 0 | 15 | 0 |
| June 21, 1912, Field | 111 | 6 | Fusarium radicicola | By inserting spores and hyphae into base of stem | Young | 100 | 1 | 155 | 1 |
| July 1, 1912, Field | 102 | 13 | Gibberella saubinetii | By inserting spores and hyphae into base of stem | Young | 100△ | 0 | 155 | 0 |
| May 27, 1912, Greenhouse | 91 | 3 | Fusarium orthoceras | By inserting spores and hyphae into base of stem | Young | 10 | 0 | 15 | 0 |
| June 20, 1912, Field | 111 | 3 | Fusarium orthoceras | By inserting spores and hyphae into base of stem | Young | 100 | 0 | 155 | 0 |
| January 11, 1913, Greenhouse | 40 | 18 | Fusarium orthoceras | By inserting spores and hyphae into base of stem | Young | 10 | 0 | 10 | 0 |
| January 11, 1913, Greenhouse | 40 | 19 | Fusarium orthoceras | By inserting spores and hyphae into base of stem | Young | 10 | 0 | 10 | 0 |

* None of the checks were infected.

† These inoculations were made by mixing conidia and ascospores in tubes of sterile water and then pouring the contents about the stem of the plants.

‡ See page 287 for explanation of results of inoculation experiments.

§ See footnote 2, Table 1.

¶ The same plants were inoculated October 3, 1912, with cultures of the same organism but none became infected.

⁂ The culture was furnished by H. W. Wollenweber, which was isolated by him from Irish potatoes received from Utah.

△ Variety "Southern Queen."

TABLE III

*Results of inoculating* various hosts† with Fusarium batatatis, F. hyperoxysporum and Nectria ipomoeae*

| HOST | DATE AND PLACE OF INOCULATION | LENGTH OF EXPERIMENT IN DAYS | ORGANISM NUMBER | NAME OF ORGANISM | NUMBER | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | INOCULATED | INFECTED | CHECKS‡ | PERCENTAGE INFECTED |
| Lycopersicon esculentum | June 18, 1912, Greenhouse | 69 | 4 | Nectria ipomoeae | 12 | 0 | 4 | 0 |
| Solanum melongena | September 3, 1912, Greenhouse | 115 | 1 | Fusarium batatatis | 5 | 0 | 5 | 0 |
| Solanum melongena | July 3, 1912, Greenhouse | 68 | 4 | Nectria ipomoeae | 10 | 0 | 6 | 0 |
| Solanum melongena | July 3, 1912, Greenhouse | 114 | 12 | Fusarium hyperoxysporum | 10 | 0 | 6 | 0 |
| Solanum melongena | October 9, 1912, Greenhouse | 134 | 12 | Fusarium hyperoxysporum | 8 | 0 | 10 | 0 |
| Solanum melongena | July 9, 1912, Greenhouse | 64 | 12 | Fusarium hyperoxysporum | 8 | 0 | 5 | 0 |
| Capsicum annuum | June 22, 1912, Greenhouse | 79 | 10 | Nectria ipomoeae | 8 | 0 | 4 | 0 |
| Capsicum annuum | July 3, 1912, Greenhouse | 114 | 12 | Fusarium hyperoxysporum | 8 | 0 | 4 | 0 |
| Solanum tuberosum | October 9, 1912, Greenhouse | 41 | 12 | Fusarium hyperoxysporum | 6 | 0 | 4 | 0 |
| Ipomoea hederacea | September 3, 1912, Greenhouse | 39 | 1 | Fusarium batatatis | 10 | 6 | 5 | 60 |
| Ipomoea hederacea | July 3, 1912, Greenhouse | 79 | 4 | Nectria ipomoeae | 10 | 0 | 9 | 0 |
| Ipomoea hederacea | July 3, 1912, Greenhouse | 79 | 12 | Fusarium hyperoxysporum | 10 | 3 | 9 | 30 |
| Ipomoea hederacea | August 8, 1912, Field | 63 | 12 | Fusarium hyperoxysporum | 12 | 9 | 5 | 75 |
| Ipomoea lacunosa | August 8, 1912, Field | 63 | 12 | Fusarium hyperoxysporum | 15 | 0 | 6 | 0 |
| Trifolium pratense | July 3, 1912, Greenhouse | 68 | 4 | Nectria ipomoeae | 5 | 0 | 5 | 0 |
| Trifolium pratense | July 3, 1912, Greenhouse | 114 | 12 | Fusarium hyperoxysporum | 5 | 0 | 5 | 0 |
| Ipomoea purpurea | October 9, 1912, Greenhouse | 87 | 1 | Fusarium batatatis | 10 | 0 | 10 | 0 |
| Ipomoea purpurea | October 9, 1912, Greenhouse | 87 | 12 | Fusarium hyperoxysporum | 10 | 0 | 10 | 0 |
| Ipomoea coccinea | November 18, 1912, Greenhouse | 95 | 1 | Fusarium batatatis | 7 | 0 | 8 | 0 |
| Ipomoea coccinea | November 18, 1912, Greenhouse | 95 | 12 | Fusarium hyperoxysporum | 8 | 0 | 8 | 0 |

* Inoculated by inserting spores and hyphae at base of stem.

† The plants were all young.

‡ None of the checks were infected.

TABLE IV

*Organisms isolated from stored sweet potatoes and the frequency of their occurrence*

| NUMBERS AND NAMES OF ORGANISMS WITH WHICH FIELD INOCULATIONS WERE MADE | PENICILLIUM SP. | NO GROWTH IN CULTURE | UNDETERMINED FUNGI | DIAPORTHE BATATATIS | RHIZOPUS SP. | MACROSPORIUM SP. | BACTERIA | FUSARIUM SP. | PESTALOZZIA SP. | ALTERNARIA SP. | F. ACUMINATUM | F. ORTHOCERAS VAR. TRISEPTATUM | F. CAUDATUM VAR. VOLUTUM | F. COERULEUM | F. SOLANI VAR. MEDIUM | F. INCARNATUM | F. BATATATIS | F. HYPEROXYSPORUM | F. OXYSPORUM | F. CAUDATUM | F. RADICICOLA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. 1, F. batatatis* | 2 | 7 | | | | | | | | | | | | | | | 8 | | | | |
| No. 1, F. batatatis† | 2 | 21 | 2 | 1 | | | | | | | 1 | | | | | | 3 | | | | |
| No. 2, F. batatatis* | 1 | 9 | | 1 | 3 | 2 | | | | | | | | | | | 10 | | | | |
| No. 2, F. batatatis† | 1 | 20 | | | 1 | 1 | 1 | | | | | 1 | | | | | 6 | | | | |
| No. 3, F. orthoceras | 8 | 2 | 7 | | | | | 1 | | | 1 | 1 | 1 | | | | | | 14 | 1 | |
| No. 4, Nectria ipomoeae | 5 | 1 | | | | 3 | | | | | | 1 | | 1 | | | | | 7 | | |
| No. 5, F. oxysporum | 12 | | | | | | | | 1 | 1 | 1 | 1 | | | | | 1 | | 8 | | |
| No. 6, F. radicicola | 11 | 3 | 2 | 2 | | 3 | | | | | 3 | 5 | | | | 3 | 1 | | 6 | 1 | |
| No. 7, F. oxysporum | 5 | 4 | 1 | | | | | | | | 3 | 3 | | | | | | | 12 | | 1 |
| No. 8, F. caudatum | 5 | 3 | 3 | | 2 | 1 | 1 | | | | 1 | 4 | | | | 1 | | | 7 | 1 | 1 |
| No. 9, F. oxysporum | 18 | 3 | 2 | 2 | 3 | 1 | | | 1 | | | 2 | | | | 1 | | | 17 | | 1 |
| No. 12, F. hyperoxysporum | 4 | | | | | | | | | | | | | | | | | 5 | | | |
| No. 13, Gibberella saubinetii | 2 | 4 | | | | 1 | | | | | | 1 | | | | | | | 4 | 1 | |
| Checks | 7 | 14 | 3 | | 1 | | | | | 3 | 2 | 3 | | | 2 | 2 | | | 23 | | |

* Plants showing brown discoloration of the fibro-vascular bundles of the stem, marked diseased when dug.

† Stems apparently not diseased when dug.

ETIOLOGY OF STEM-ROT

Our investigations lead us to the conclusion that the stem-rot of *Ipomoea batatas* is caused by either *F. batatatis* or *F. hyperoxysporum*, and not by *Nectria ipomoeae*. The symptoms produced by the two parasitic species are the same. The morphological and physiological differences between the two are clearly set forth by Wollenweber (1914).

There is but little doubt, judging from Halsted's writing (1890), but that we have been studying the same disease that he had under observation in 1890. Two years later (Halsted, 1892) he came to the conclusion that *Nectria ipomoeae*, was the cause of the stem-rot of the sweet potato and eggplant, and in 1894 (Halsted, 1895) concluded that the ring-rot (pl. XVI) of the sweet potato was also caused by the same fungus. *Nectria ipomoeae* is a rather cosmopolitan species and commonly found associated with decayed sweet potatoes and eggplants. We have frequently found it in storage houses, hot beds, and on dead vines in the field during the summer, but neither eggplants nor sweet potatoes inoculated with the organism ever developed symptoms characteristic of stem-rot. Frequent attempts to find a Fusarium parasitic on *Solanum melongena* have been unsuccessful. We have several times isolated *Verticillium albo-atrum* from the fibro-vascular bundles of eggplants from New Jersey, and it is barely possible that this is the disease attributed by Halsted to *Nectria ipomoeae*, which is frequently found on dead or partially dead eggplant stems.

As already pointed out, we have isolated two species of Fusarium which produced the characteristic symptoms of stem-rot when inoculated into sweet potatoes. It may be urged that these two species are but conidial forms of *Nectria ipomoeae*. Halsted showed that it was easy to produce the ascogenous form from the conidia and the conidia from ascospores in pure culture. We have obtained similar results, thus confirming the work of Halsted. We have grown hundreds of cultures of *F. batatatis* and *F. hyperoxysporum* and in no case has anything suggesting perithecia ever been developed.

The morphological differences between the conidial stage of *Nectria ipomoeae* on the one hand and *F. batatatis* and *F. hyperoxysporum* on the other also tend to prove that the two latter are not imperfect stages of Nectria. The conidia of *F. batatatis* (fig. 2, *C*) and *F. hyperoxysporum* (fig. 2, *G*) are mostly 3-septate, rarely 4- and 5-septate, and measure 25–45$\mu$ long and 2.75–4$\mu$ wide; 4- and 5-septate conidia measure 37–50$\mu$ long and 3–4$\mu$ wide, while the conidia of *Nectria ipomoeae* (fig. 2, *D*) are mostly 5-septate and measure 30–70$\mu$ long and 3.75–5.5$\mu$ wide. They are therefore much larger. They differ also in shape and in character of growth in

artificial cultures. For additional differential characters, the reader is referred to the recent works of Wollenweber (1913 and 1914).

### RELATION OF THE STEM ROT ORGANISMS TO STORAGE ROTS

*Experiment 1.* In October, 1912, 200 diseased sweet potatoes were selected from a field in New Jersey. No cultures were made at the time the potatoes were selected, the blackened bundles of the potatoes being regarded as evidence of disease. These potatoes were put in a bushel basket and stored in the usual way in a storage house on the farm where the potatoes were collected. The following spring the potatoes were shipped to Washington, D. C., and cultures made from each potato and the species determined, if a Fusarium. When the cultures were made, 181 potatoes were sound and 19 rotten or partially so. The organisms isolated from the sound potatoes were identified as follows: *Fusarium batatatis*[4] 140; *F. oxysporum* 5; *F. orthoceras* var. *triseptatum* 3; *F. incarnatum* 1; *F. acuminatum* 1; *F. caudatum* var. *volutum* 1; *Penicillium* sp. 10; *Rhizopus* sp. 9; no growth in culture 7; undetermined fungi 4. From the decayed or partially decayed potatoes the organisms were identified as follows: *F. batatatis* 3; *Penicillium* sp. 8; *Rhizopus* sp. 7; undetermined fungus 1.

*Experiment 2.* On October 29, 1913, 50 sweet potatoes were inoculated (25 at the end and 25 at the side) with *Fusarium hyperoxysporum* after disinfecting for ten minutes in a solution of mercuric chloride (1–1000) and rinsing in sterile water. Each potato was then wrapped in oiled paper, placed in a cloth bag, and stored in the potting shed of the greenhouse. Fifty other potatoes were inoculated on October 31 with *Fusarium batatatis* (25 at the end and 25 at the side) but otherwise treated as above. All these potatoes were examined on January 5 and none were decayed.

*Experiment 3.* On January 3, 1914, lots of 10 sweet potatoes inoculated at the end with *Fusarium batatatis*, and lots of 10 inoculated with *Fusarium hyperoxysporum* were placed in thermostats 3, 4, 5, 7, 9, 10, 11 and in moist chambers in the laboratory room. Ten potatoes were held as checks. The incubators[5] maintained at the lower temperatures were always moist, water of condensation invariably being present on the walls. This experiment was terminated January 26. The temperatures during the storage period are shown in the following table:

[4] In some cases where there was doubt as to whether the species was *batatatis* or *oxysporum*, the point was settled by inoculations in the greenhouse.

[5] Temperatures in incubators 3–10 were maintained by icing, in number 11 by electric bulbs.

TABLE V

*The minimum, maximum and average temperature of the different thermostats and laboratory room during the storage period*

*Thermostat*

| | TEMPERATURE °C.* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 7 | 9 | 10 | room | 11 |
| Minimum | 6.7 | 8.0 | 11.3 | 16.3 | 18.0 | 19.0 | 19.0 | 26.3 |
| Maximum | 9.8 | 11.1 | 14.5 | 19.9 | 21.8 | 22.2 | 25.5 | 28.4 |
| Average | 8.3 | 9.1 | 13.2 | 18.5 | 20.3 | 21.0 | 22.3 | 27.3 |

* Temperatures read about 9.15 a.m. and about 4.15 p.m. each day except Sunday.

Results of inoculations with *Fusarium batatatis*. No potatoes in incubators 3, 10, 11, in the laboratory room, and checks were decayed. In incubator 4, three were decayed (unidentified); in 5, two (*Rhizopus* sp. and *Fusarium* sp.); in 7, five (*Fusarium batatatis* 3, *Rhizopus* sp. 1, *F. batatatis* and *F. incarnatum* 1); and in 9, two (*Fusarium solani* and *F. batatatis*). Results of inoculations with *Fusarium hyperoxysporum*. No potatoes in incubator, 3, 5, 7, 11 and laboratory room were decayed. In incubator 9 one was decayed (*Fusarium hyperoxysporum*) and in incubator 10, 1 (*Rhizopus* sp.).

*Experiment 4.* On January 26, 1914, lots of six selected stem-rot sweet potatoes as determined by the blackening of the fibro-vascular bundles[6] were placed in incubators 3, 5, 7, 9, 10 and in moist chambers in the laboratory room. Saturated sponges were kept in the incubators held at the higher temperatures but this procedure seemed in no way to affect the results. The potatoes were disinfected in a solution of mercuric chloride (1–1000) for ten minutes then rinsed in sterile water. They were not inoculated. The experiment was terminated February 24. During the storage period the temperatures in the incubators and laboratory are shown in the following table.

Results. In incubators 3, 5 and 10 none of the potatoes were decayed. In laboratory room one potato rotted and after isolation the organism was identified as *Rhizopus* sp. In incubator 7, one potato was about one-third decayed and another just started. The organisms from both were identified as *Fusarium batatatis*. In incubator 9 two potatoes were partially rotted and the organisms were identified as *Diaporthe batatatis* and *Nectria ipomoeae*.

[6] About half of these potatoes was selected in the field during the fall of 1913 at digging time. Only potatoes with blackened bundles were selected from diseased vines. The other half was selected by G. A. Mitchell from a house at Vineland, New Jersey, in which stem rot potatoes had been stored.

TABLE VI

*Showing the minimum, maximum and average temperatures of the different thermostats and laboratory room during the storage period*

*Thermostat*

| | TEMPERATURE °C.* | | | | | |
|---|---|---|---|---|---|---|
| | 3 | 5 | 7 | 9 | 10 | room |
| Minimum | 5.3 | 9.1 | 12.2 | 13.5 | 14.0 | 21.5 |
| Maximum | 10.2 | 15.1 | 19.8 | 21.8 | 22.5 | 26.0 |
| Average | 7.8 | 13.0 | 17.3 | 19.2 | 19.9 | 23.5 |

* Temperatures read about 9.15 a.m. and 4.15 p.m. each day except Sunday.

Within the limitations of these experiments the results indicate that the stem-rot organisms do not cause storage rots or at least only rarely so, and then only under most favorable conditions. Final conclusions on this point will be withheld, however, until additional experiments have been conducted, the results of which will be published in an article on the "Storage rots of the sweet potato."

## DISSEMINATION

A disease of this type, the spores of which live over winter in the soil, may be distributed by such agencies as (1) insects, (2) farm animals which are allowed to roam from one field to another, (3) farm implements, (4) drainage water, (5) wind, (6) with the slips themselves, and (7) stable manure and hot bed soil.

In New Jersey, where the soil is light and sandy, the spores are undoubtedly carried from place to place by strong winds which raise clouds of dust during certain seasons of the year. This is the only explanation which can be offered to account for infection in one field under observation at Vineland, New Jersey, which had not been planted to sweet potatoes for forty years or more, and yet the first year gave 25 to 50 per cent of diseased plants. It might be urged that the disease was carried there with the slips. That, however, was not likely since the potatoes used to produce the slips were grown under the best possible conditions. The potatoes were selected in the field the previous fall from healthy hills and carefully protected during the winter. The soil for the hotbed was either treated with formalin or secured from beneath the surface after throwing off several inches of the top layer. Several diseased sweet potato fields were located nearby, so that it seems most likely that the soil became infected as the result of strong winds.

It was generally believed by many growers, and the writers were of the same opinion, that the disease had its origin to a considerable extent in the hotbed. It was believed that diseased potatoes were carelessly selected for seed and that these always gave diseased sprouts. Our experiments show that diseased potatoes do give diseased sprouts but not to the extent that might be expected. For example, out of twenty-three diseased potatoes bedded in one experiment, only one gave diseased slips—twelve in all.

On May 15, 1913, another experiment was started, in which 75 selected stem-rot infected potatoes were bedded. On July 16, 320 plants were drawn and so far as could be judged from a superficial examination, none had stem-rot. On August 8, 77 plants were drawn and 14 had stem-rot without question. On September 2, 54 more plants were drawn and 4 had stem-rot. It is probable that many of the plants that seemed to be free from disease would have developed stem-rot if they had been set out and allowed to grow for a few weeks. In fact, we have often observed in other connections that plants apparently disease-free when drawn were in reality diseased and would develop the disease later when set out in pots, the soil of which had been steamed. It is suggested from our experiments and generally believed by farmers that the first draw is less likely to be diseased than the second and third. In fact, in the experiment just mentioned, not a single plant of the first draw was diseased when pulled so far as we could tell. On the other hand, in another experiment all the plants on one potato had stem rot.

Farmers frequently exchange seed or sell slips and sometimes send them long distances. Therefore, in view of the fact that the disease resides both in the potato and slips, it is likely that the sale of potatoes and slips is largely responsible for the wide distribution of the stem-rot organism.

## REMEDIAL MEASURES

No sure method for the control of this disease is yet known. In view of the general infestation of the soil by these organisms and the prevalence of field infections, it is doubtful if a complete control or eradication can be accomplished unless resistant varieties can be found that are suitable for growing commercially in such localities. Experiments to determine the comparative resistance of different varieties to stem-rot, while not completed, indicate that some are entirely or very resistant and others particularly susceptible to the disease. The phase of the work will be published later as a separate article.

Remedial measures should consist in the careful selection of seed in the fall from healthy vines. When selecting seed it is advisable to split

open the stem, and if the fibro-vascular bundles are blackened, the plant is probably diseased and no potatoes should be taken from the hill. Selected seed potatoes should be disinfected before bedding by soaking for two minutes in a solution of corrosive sublimate (1–1000). Disease-free soil should be used in the hotbed and the material used in its construction should be previously sprayed with Bordeaux mixture or some good disinfectant. By practicing such remedial measures the writers have been able to materially increase the yield even on badly infected soil.

## SUMMARY

1. Sweet potato stem-rot is caused by either one of two species of Fusarium, viz. *F. batatatis* or *F. hyperoxysporum*.

2. The stem-rot organisms are vascular parasites, invading the bundles of all parts of the plant, producing a brown discoloration.

3. Both species have been successfully inoculated into *Ipomoea hederacea*, the wild ivy-leaved morning glory.

4. The stem-rot organisms are not parasitic on eggplants, tomatoes, peppers, clover, Irish potatoes, *Ipomoea purpurea*, *I. coccinea* or *I. lacunosa*.

5. All attempts to produce stem-rot of sweet potato and eggplant with *Nectria ipomoeae* have been unsuccessful.

6. *Nectria ipomoeae* is commonly found on rotting sweet potatoes in storage.

7. The following species of Fusarium which were isolated in connection with storage rots were inoculated into living sweet potato plants in the field, but without success: *F. oxysporum*, *F. orthoceras*, *F. caudatum* and *F. radicicola*. Inoculations with *Gibberella saubinetii* and *Nectria ipomoeae* were also unsuccessful.

8. Heavy losses are incurred through stem-rot in some localities, notably New Jersey and Delaware.

9. Stem-rot results in loss of stand and a decreased yield from those hills which have survived, although infected.

10. The majority of infections take place in the field, though the organisms may grow from diseased potatoes into the slips produced therefrom.

11. The organisms may be distributed with seed potatoes and slips, and by wind, insects, farm implements, etc.

12. The disease may be lessened through careful selection of seed and sterilization of the seed bed.

BUREAU OF PLANT INDUSTRY
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LITERATURE

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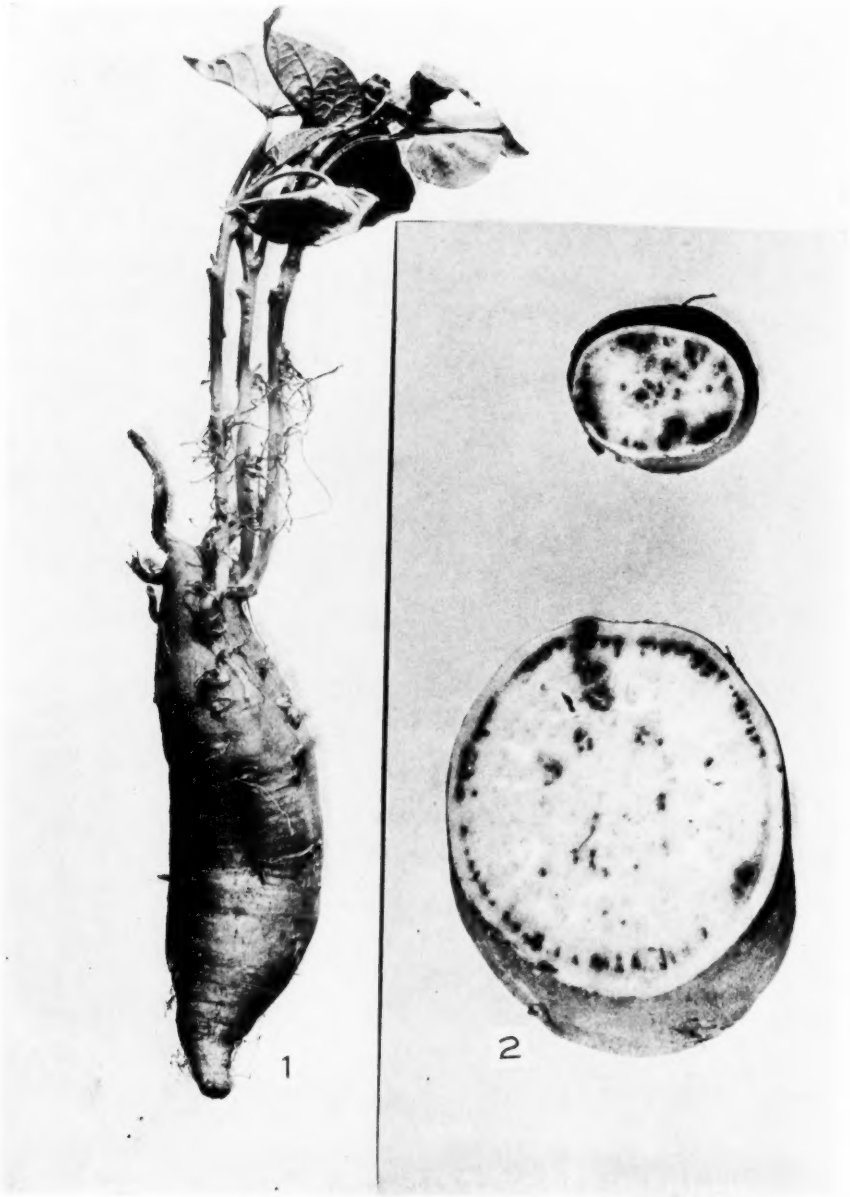
HARTER AND FIELD: STEM ROT OF THE SWEET POTATO

A hill of sweet potatoes attacked by the stem-rot, showing the different stages in the progress of the disease.




HARTER AND FIELD: STEM ROT OF THE SWEET POTATO

FIG. 1. Diseased slips growing from an infected seed potato.

FIG. 2. Cross section of a sweet potato showing the characteristic blackening of the fibro-vascular bundles, produced by stem-rot.

HARTER AND FIELD: STEM ROT OF THE SWEET POTATO

Ring rot of sweet potatoes which was formerly thought to be caused by *Nectria ipomoeae*. The sunken portion may occur any place between the ends of the potato.

# RECENT STUDIES OF SOME NEW OR LITTLE KNOWN DISEASES OF THE SWEET POTATO[1]

J. J. TAUBENHAUS

WITH PLATES XVII, XVIII, AND XIX

## CHARCOAL ROT

*Sclerotium bataticola* Taub.

In a recent paper on the black rots of the sweet potato[2] the writer has described the charcoal rot due to *Sclerotium bataticola* Taub. and has also proven it to be distinct from the black rot produced by *Sphaeronema fimbriatum* (E. & H.) Sacc. of Halsted. The charcoal rot is the less familiar of the two to plant pathologists and its fungus nature is unknown to the sweet potato grower, who usually attributes it to heat, scalding or freezing in the storage house. The disease is of considerable economic importance. It is most abundant in overheated houses which are maintained from 80 to 90° F. during sweating, and in bins which are nearest or next to the stoves, the loss varying from 2 to 15 per cent. The typical rot is obtained when *Sclerotium bataticola* is the only invader. In this case the infected root in the last stages of decay presents internally a charcoal appearance, the substance being more or less friable when dry. It often happens that the fungus *Sclerotium bataticola* is followed by *Fusarium batatis* Woll. When both fungi are present it matters not which of these is the primary invader, and the affected root does not turn charcoal but takes on an ashen color which varies with the predominating fungus. Quite often, a root infected with *Fusarium batatis* shows also, upon a close macroscopical examination, the presence of the minute sclerotia of *S. bataticola* scattered very sparsely in the tissue, but here the ashen color is not even present.

Recent studies on this disease seem to indicate that infection takes place at a bruise on the epidermis and from there the fungus works slowly inwards. It is not uncommon, therefore, in light cases of infection, to find, upon making a cross section of the root, a jet black ring ranging from $\frac{1}{3}$ to $\frac{1}{2}$ inch in diameter immediately under the epidermis, the color grading off from dark to light ash as it nears the center of the root. At

[1] Paper read before the American Phytopathological Society at the Atlanta Meeting, January, 1914.

[2] Taubenhaus, J. J. The black rots of the sweet potato. Phytopathology **3**: 159–166. 1913.

this stage the infected sweet potato is water-soaked but quite solid. A liquid, more or less brown in color, may be squeezed out from such roots. Charcoal rot is almost unknown in well-ventilated houses. There seems no doubt but that the fungus *S. bataticola* is a common field saprophyte and may be brought in to the storage house with the sand or soil which clings to the roots. I have often found the fungus in the field on dead sweet potato stems which were killed by *Fusarium batatis* Woll. In overheated and ill-ventilated houses *S. bataticola* is very often found to follow the soft rot caused by *Rhizopus nigricans* Ehr. In this case, the symptoms are distinguished from typical charcoal rot only by the fact that the roots here are soft and mushy, a condition characteristic of soft rot.

### SOFT ROT AND RING ROT

### *Rhizopus nigricans* Ehr.

It is difficult to describe ring rot without first considering soft rot, as the two diseases are caused by the same fungus.

*Historical.* Halsted[3] was the first (1890) to attribute soft rot of the sweet potato to the fungus *Rhizopus nigricans* Ehr. In 1892 he again called attention to a quince rot[4] which he attributed to the same fungus. From 1890 to 1909 all the writers on sweet potato diseases quoted Halsted verbatim. In 1909 Orton[5] called attention to a decay of the white or Irish potato which is generally known as "leak" or "Melters," and which he attributed to *Rhizopus nigricans* Ehr. In 1909, Morse[6] mentioned a fruit rot caused by a species of Rhizopus. Haslam[7] (1910) found the fungus *Rhizopus nigricans* in great abundance on corn, producing blind staggers in horses. Edgerton[8] (1911) described a soft rot of the fig which he attributed to the same fungus. He claimed in some cases a loss of more than 90 per cent of the fruits. Finally, Blakeslee and Gortner[9] (1913)

[3] Halsted, B. D. Some fungous diseases of the sweet potato. N. J. Agr. Expt. Sta. Bul. 76: 3–31. 1890.

[4] Halsted, B. D. Some fungous diseases of the quince fruit. N. J. Agr. Expt. Sta. Bul. 91. 1892.

[5] Orton, W. A. Potato diseases in San Joaquin County, California. U. S. Dept. Agric. Bur. Plant Indus. Circ. 23, p. 14. 1909.

[6] Morse, W. J. Notes on plant diseases in 1908. Maine Agr. Expt. Sta. Bul. 164: 1–28. 1909.

[7] Haslam, Th. P. Meningo-Encephalites (Blind staggers). Kansas Agr. Expt. Sta. Bul. 173: 235–251. 1910.

[8] Edgerton, C. W. Diseases of the fig tree and fruit. La. Agr. Expt. Sta. Bul. 126: 3–20, 1911.

[9] Blakeslee, A. F. and Gortner, R. A. On the occurrence of a toxin in juice expressed from the bread mould, Rhizopus nigricans (Mucor stolonifer). Biochem. Bul. **2**: 542–544. 1913.

announced the discovery of a toxin secreted by *R. nigricans*. This is so virulent that "when a dose containing the toxin from a greater quantity than 0.045 gm. is injected, the animal (rabbit) is often dead before the needle can be withdrawn."

*Occurrence.* Our observations verify those of Halsted and others that soft rot is mostly a storage trouble, although it is sometimes met with in the field at digging time and in the seed bed. With sweet potatoes it is constantly associated with poorly ventilated houses and even the best ventilated houses are not entirely free from it. In Delaware it causes more damage to stored sweet potatoes than all the other diseases combined. Yet farmers insist they are concerned less about soft rot than black rot. On an average fully 30 per cent of the total crop in storage is lost from diseases in Delaware, and this loss 20 per cent can be safely attributed to soft rot. In Alabama Wilcox[10] and Carver[11] estimate that soft rot is by far the most important of the diseases which affect sweet potatoes in storage. It is very likely that this condition prevails in other states where sweet potatoes are stored. The reason why storage men and pathologists overlook soft rot will be considered later.

*Symptoms of soft rot.* The term soft rot best describes the character of this disease. Affected roots are very soft, allowing ready penetration of the finger. A soft-rotted root is water-soaked and when pressed, a clear liquid readily oozes out. In storage, the disease may be detected by the wetting of the adjacent healthy roots. Under storage conditions, Rhizopus-infected roots do not produce sporangia unless broken or bruised. Such breaks are often produced by the weight of unrotted roots above. Where this occurs, a black mass of short-stemmed sporangia is formed at the crack, through which opening the liquid drips out. When no cracks are formed, the fungus fails to fruit and such roots dry through gradual evaporation. Soft rot first takes place in storage as the roots undergo sweating, which usually requires three to four weeks. As a rule, the potatoes are not disposed of before they have been in storage from four to eight weeks. By that time, all the soft-rotted potatoes have thoroughly dried, and as a result are mistaken for dry rots. As is often the case, in storage the soft-rotted roots do not produce the fruit of the fungus; thus farmers and storage men fail to recognize the disease, and hence underestimate the damage caused by it.

[10] Wilcox, E. M. Diseases of sweet potatoes in Alabama. Ala. Agr. Expt. Sta. Bul. 135: 3–15. 1906.

[11] Carver, G. W. Saving the sweet potato crop. Agr. Expt. Sta. (Tuskegee, Ala.) Bul. 10: 5–14. 1906.

Carver, G. W. Possibilities of the sweet potato in Macon County. Ibid., Bul. 17: 5–19. 1910.

*Pathogenicity.* None of the authors above mentioned reported any experiments to prove the pathogenicity of *Rhizopus nigricans.* It is generally agreed that poorly-ventilated houses, especially during sweating, greatly favor the trouble. In order to determine the pathogenicity of the fungus, and also to learn whether it is the lack of fresh air or the excess of moisture in the poorly-ventilated houses that favors soft rot, the following experiments were carried out.

*Moisture experiment with crude sulphuric acid.* Bell jars were used to cover wire baskets 5 x 5 x 5 inches containing sound sweet potatoes. These were kept in the laboratory one week to determine the presence or absence of soft rot infection. Crude sulphuric acid was used as a drying agent, being placed in flat glass dishes 5 inches in diameter and 2 inches deep. The amount of acid used is indicated in table 1. Two of the baskets were left as checks without the drying agent; the remaining six were subjected to the acid. Duplicates marked A and B in table 1 were run in each experiment. Before covering with the bell jars each root in the baskets was smeared with spores from pure cultures of *R. nigricans,* applied with a camel's hair brush. The results obtained are given in table 1.

From table 1 it is seen that both checks were infected with soft rot the third day of the experiment, giving an average of 85 per cent infection. Where 100 cc. of sulphuric acid was used infection was retarded from

TABLE 1*

| KIND OF TREATMENT | CHECKS | | 100 CC. ACID | | 50 CC. ACID | | 25 CC. ACID | |
|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B |
| Weight in grams of sweet potatoes at first date of experiment | 1363 | 1362 | 1465 | 1347 | 1363 | 1290 | 1360 | 1307 |
| Weight in grams of same at end of experiment | 1243 | 1195 | 1077 | 1056 | 1135 | 1040 | 1132 | 1090 |
| Date of inoculation | Dec. 5 12 | Dec. 5 12 | Dec. 5 12 | Dec. 5 12 | Dec. 5 12 | Dec. 5 12 | Dec. 5 12 | Dec. 5 12 |
| First date of infection | Dec. 8 12 | Dec. 8 12 | Jan. 12 13 | Dec. 28 12 | Dec. 20 12 | Dec. 21 12 | Dec. 15 12 | Dec. 16 12 |
| Per cent of infection | 90% | 80-85% | 7% | 7.7% | 83% | 80% | 80% | 90% |
| Amount of water given off in grams | 120 | 167 | 388 | 291 | 228 | 250 | 228 | 217 |

*Variety used, Up River

twenty-three to thirty-eight days and resulted in only 7 per cent of infection (pl. XVII, figs. 4 and 5). As the acid was reduced from 100 to 50 cc., infection was retarded from fifteen to sixteen days, and increased to about 80 per cent. As the acid was further decreased to 25 cc. infection was retarded ten to eleven days, and infection increased from 80 to 90 per cent, nearly equaling the per cent of infection in the checks. It is evident, therefore, that the more acid we use the greater its power of absorbing the moisture which is given off and therefore the greater the time required for infection. The contrary occurs as we decrease the amount of acid, in which case the amount of acid is insufficient to absorb the moisture as rapidly as it is given off by the sweet potatoes. It was further noticed that in the checks where there was no acid to absorb the moisture, drops of water were seen to collect on the walls of the bell jars, whereas none were present on the walls of the bell jars containing acid. However, in the weaker strengths of the acid, after a week or ten days, drops of water collected on the walls, particularly at night. These drops disappeared during the day. Infection in these cases no doubt took place at night, because of the moisture which prevailed. With the stronger amounts of acid used, the moisture given off was readily absorbed but a point was reached here, also, where the acid became too dilute to absorb the moisture given off. A few drops of water were later noticed on the walls of the bell jars and infection started. This experiment clearly demonstrated that it is the presence of moisture and not the lack of fresh air which permits *Rhizopus nigricans* to produce soft rot, for at no time during the entire run of the experiment were the bell jars lifted to introduce fresh air. The above experiment was repeated in 1913, using this time chemically pure sulphuric acid. The results obtained were similar to those reported above.

*Experiments with open versus closed moist chambers.* In order to further test whether lack of ventilation or presence of moisture is favorable to soft rot, the following experiment was tried. Healthy sweet potatoes were selected and kept in the laboratory for one week. The healthy roots, five of each, were placed in flat sterile moist chambers, ten in all. Five were marked A1, A2, A3, A4 and A5, and these were used as checks. The other five were marked B1, B2, B3, B4 and B5. On December 20, 1913, the sweet potatoes in all the moist chambers were inoculated with spores from pure cultures of *Rhizopus nigricans* Ehr. The method of inoculation was the same as described under Experiment 1. Soon after the inoculation, chambers of the A series were left open, chambers of the B series were covered. On December 21, 1912, the walls of the chambers of the B series were seen covered with drops of water. The roots too in these were moist. Moreover, the roots in chambers B2 and B5 were soft-

rotted.[12] All the roots in the A series were dry and healthy. On December 22, 1912, the sweet potatoes in moist chamber B1 were all soft-rotted (pl. XVII, figs. 2 and 3). In B2 four were soft-rotted: in B3 two were soft-rotted; in B4 and B5 all were soft-rotted, showing the typical sporangiophores of *Rhizopus nigricans*. The chambers of B series were discarded on that same date, it being proven that moisture in the closed chambers favored the development of soft rot. The A series were kept for six weeks and all but a single root during the sixth week turned soft rot. It is difficult to understand why this single check soft-rotted, unless some accidental bruise occurred.

*Experiment on cut sweet potatoes.* This experiment did not differ from No. 2 except that the roots in the moist chambers were cut in halves. Inoculations were made with fresh *R. nigricans* spores from pure cultures on January 4, 1913. The method of inoculation was the same as described under Experiment 1 with the difference, however, that the spores were applied directly on the cut surfaces of the roots Five chambers, marked C1, C2, C3, C4 and C5 were used as checks. In these the inoculated roots were placed with the cut sides up and the chambers left open. Five other chambers were marked D1, D2, D3, D4 and D5. In these, too, the inoculated roots were placed cut side up, but the chambers were covered. The experiment was begun January 4, 1912, at 5 p.m. By January 5 at 8 a.m. all the roots in the D series were soft-rotted and covered with large tufts of the mycelium and sporangia of *R. nigricans*. The roots in the C series were all healthy. The chambers in D series were discarded and those of C series retained. By January 12, all the roots in the uncovered chambers became soft-rotted and on January 13 the open cut side was covered with a black mass of tiny erect sporangiophores with mature sporangia and spores. The results obtained from this experiment confirmed the two preceding ones, namely, that moisture or a cut or bruise seem to be the governing factors for soft rot. In a cut root the place of injury seems to furnish some moisture which in time the spores may utilize in their germination.

*Resistance to soft rot.* While soft rot causes the greatest damage to sweet potatoes in storage, not all the roots are alike susceptible to the attack of *Rhizopus nigricans*. There is not only a difference in resistance of individual roots of the same variety housed under the same conditions, but there is also a difference in the ability of the roots to retain their resistance for a shorter or longer time. There is always a certain per cent of the crop, when housed poorly, that will become soft-rotted as soon as taken into the storage house. Other roots seem to possess a certain

[12] Whenever the term soft rotted is mentioned here, it is understood as being the typical rot induced by Rhizopus nigricans Ehr.

degree of resistance. These will usually keep for a month or two and then become soft-rotted, particularly if the winter is mild and the roots undergo what is known as the second sweat. There is a third class of roots which seems to be resistant and which maintains this property for a long time. These roots, although exposed to ideal conditions for infection, fail to take the disease. In the laboratory this condition is often met with. With some roots, all that is necessary to induce infection is to smear them over with spores of *R. nigricans* and then place them in a moist place. With others, neither moisture nor puncturing, nor inserting bits of mycelium in the tissue, or even placing the root near a diseased one, will induce infection. Such highly-resistant roots, when kept long enough in storage or in the laboratory will produce a number of buds or sprouts. It seems that such roots possess a higher enzyme activity, a condition unfavorable to the fungus.

*Fruiting conditions of R. nigricans.* Extended studies of storage house conditions, together with laboratory experiments, seem to establish the following facts: Sweet potatoes both in storage and in the laboratory, if infected with soft rot will not produce Rhizopus fruits as long as the epidermis is unruptured and left exposed to the open air. In this case the infected root turns soft but in time loses all its water through evaporation and shrivels, becoming very hard (pl. XVII, fig. 7) When the epidermis of these same infected roots is broken while soft, a black tuft of short-styled and closely packed sporangia of *R. nigricans* is soon formed at the opening (pl. XVII, fig. 10). On the other hand, when infected but uninjured roots are placed in a closed moist chamber, the fungus, in twelve hours, sends out tufts of mycelium with sporangia (pl. XVII, fig. 1). When a freshly-infected sweet potato with soft rot is cut in halves and allowed to be exposed in open air, the cut surface will in twelve hours be covered with small, erect, short-styled sporangiophores. If, after this, it is again placed in a closed moist chamber, it will, after six to twelve hours, send out large tufts of mycelium with sporangia. It seems evident, therefore, that either a break in the epidermis or a saturated atmosphere are governing factors in the fruiting of *R. nigricans* upon sweet potatoes.

*Odor of soft rot.* We very often hear storage men accuse the soft rot of emitting strong disagreeable odors. Similar statements are also found in the literature. Price[13] writes as follows: "The potato when attacked, at once becomes soft and worthless and gives off an offensive odor." Starnes[14] too has the following to say: "The entire contents of a bin or hill is reduced to a pulpy mass of corruption, emitting a most disgusting

[13] Price, R. H. Vegetables. Texas Agr. Expt. Sta. Bul. 36: 611–625. 1895.

[14] Starnes, H. N. Sweet potato. New Encyclopedia of American Horticulture by L. H. Bailey **6**: 1754–1755. 1906.

odor." My own observations and studies show that soft rot in the bins is odorless for a week or ten days. After that time acetic fermentation sets in. The odor of fermentation then is quite noticeable. It seems that this odor attracts the females of a species of fruit fly which lay their eggs in the rotting potato. At this stage badly affected storage houses are literally swarming with these flies. The acetic fermentation may last for a week to ten days. After that time, the affected potatoes have fairly dried out and other fungi, such as *Diaporthe batatis* (Hals.) Hart., *Fusarium batatis* Woll., *Sclerotium bataticola* Taub., and a number of other fungi may gain entrance. It sometimes happens that putrefaction follows the acetic fermentation. Bacteria are no doubt involved in this process, for at this time the affected potato is fairly putrid and the odor is erroneously attributed to soft rot.

*Longevity of the Rhizopus mycelium in the affected host.* I have previously stated that sweet potatoes affected with soft rot may only produce the sporangia of the causative fungus when the roots are bruised or placed in a closed moist chamber. Therefore, it became necessary to determine how long the mycelium of *R. nigricans* may remain alive in the affected roots. A large number of sweet potatoes was secured. These were infected with soft rot, the infection being twenty-four hours old. The infected roots showed no sporangia. Such roots may easily be secured by placing healthy sweet potatoes in a closed hamper in a warm room, where nearly 50 per cent of the roots will become soft-rotted. The infected roots were handled separately to avoid injury and they were placed separately in open flat chambers to prevent the fruiting of the fungus. Platings were made after surface sterilization by taking out blocks of tissue from the affected roots and making poured plates of nutrient agar or by crushing these blocks first with the media in the tubes and then pouring the entire contents into a petri dish. This is a modified method adopted from Manns.[15] Platings made from the interior of infected tissue of soft-rotted potatoes, one to eight days old, yielded a pure culture of *Rhizopus nigricans* (pl. XVII, fig. 8). Each day when the platings were made, two infected roots were used at a time. Nearly the entire content was cultured, making a total of thirty to fifty poured plates each day. From the ninth to the twelfth day, the Rhizopus fungus was seen to rapidly lose in vigor. At this stage, the platings yielded a very weak growth of Rhizopus followed by a species of Oospora and a species of Oosporoidea, determined by Mrs. Flora Patterson as *Oospora lactis* (Fres.) Sacc. and *Oosporoidea lactis* (Fres.) Sums, respectively. By the twelfth to fifteenth day, the fungus *R. nigricans* had disappeared completely and, instead, pure cul-

[15] Manns, T. F. Studies in diseases of cereals and grasses. Ohio Agr. Expt. Sta. Bul. 203: 213. 1909.

tures of *Oospora lactis* and *Oosporoidea lactis*, as well as bacteria, took its place (pl. XVII, fig. 9). By the fifteenth to twenty-fifth day the above two fungi had gradually disappeared, giving place to more colonies of bacteria, several species of Fusaria, *Trichoderma Köningi* Oud., *Nectria ipomeae* Hals. and several others. Microscopic examination of crush mounts on the respective dates when the platings were made, revealed some very interesting facts. During the first week, when the infected roots yielded pure cultures of *R. nigricans*, the hyphae under the microscope were hyalin and the protoplasmic granules very even. As the fungus gradually began dying, the mycelium as well as the protoplasmic granules turned brown. Many filaments too were found empty and shriveled, altogether indicating actual death of the fungus. It was further observed in studying the crush mounts, that the fungus is both inter- and intra-cellular. The middle lamella of the cells was in most part dissolved and the starch grains were very much corroded.

### RING ROT

The above consideration of soft rot has now prepared us for a better understanding of the ring rot.

*Historical.* The only mention we have of this disease is by Halsted[16] who speaks of it as follows: "The ring rot fungus (*Nectria ipomoeae* Halst.) has been investigated since the publication of bulletin 76. It proves to be caused by the same fungus as the stem rot, and is in fact a special case of the latter when the seat of the disease is at some point midway of the two ends of the root. The most striking case is when the decay takes the form of a ring about midway of the root. All over the decayed and shriveled parts, a pinkish fungus growth is found consisting of nearly spherical bodies that contain many sacs in which spores are borne. This is the same as the stem rot of the egg plant." That Halsted had in mind the same ring rot disease which we are here to consider, there is no doubt. His figures clearly indicate this. However, our finding as to the cause of the disease are at variance with his, for we find it related to the soft rot and caused by the same fungus, viz., *R. nigricans*.

*Economic importance.* Ring rot seems to be a storage disease only. The trouble is common wherever soft rot *Rhizopus nigricans* is prevalent. My studies of this disease during the current year extended to some sixty storage houses over three states, viz., Delaware, New Jersey, and Maryland. Not a single one of these storage houses was free from the disease. The per cent of loss ranged from 1 to 20 per cent. This seems also to be true for the soft rot. Sweet potato growers are so accustomed to ring

[16] Halsted, B. D. Sweet potatoes. N. J. Agr. Expt. Sta. Ann. Rept. **15**: 359–360. 1894.

rot in storage that they take it as a matter of course and make as much allowance for it as they would allow for shrinkage. That this disease should be so prevalent and yet have attracted no attention from workers is a surprise.

*Symptoms.* Before describing the symptoms, it must be stated at the outset that there are two forms of ring rot, both of which are stages of the same disease. The first stage is the soft ring. This is characterized by a soft-rotted area which girdles the root, thereby forming a complete ring. This ring may be formed in the center, at the tip end, or at any part of the root. The formation of the soft ring, like soft rot, takes place as soon as the sweet potatoes are put in storage. As the roots are usually stored in bulk in bins, and as the soft ring, like the soft rot, is confined to roots more or less deeply buried in the bin, the disease is overlooked. The soft ring, like the soft rot, is at first odorless, but in a week to ten days it is followed by a characteristic fermentation. Fruit flies which are constantly associated with soft rot in storage are also associated with the soft ring. The soft ring gradually begins to dry by losing the water which resulted from the breaking down of the starch. The drying is noticed by shrinkage and contraction of the entire ringed area, thus forming a slight groove. In two to six weeks the ring becomes very dry and more or less hardened, varying with the nature of the fungi which act as secondary invaders. This then brings us to the second form or dry ring rot, which is nothing more than the last stage of the soft ring to which the primary parasite has died out (pl. XVIII, figs. 13–19).

*Cause of the ring rot.* I have already mentioned that Halsted attributes *Nectria ipomoeae* as the cause of the ring rot. He came to this conclusion because he found the Nectria stage associated with the dead tissue. In 1911 Dr. Mel. T. Cook and the writer[17] found *Trichoderma Köningi* associated with ring rot. *T. Köningi* was proven to be an active parasite but unable to reproduce the disease. It must be added, however, that work was then being done on the dry ring rot stage in which the primary parasite had long died out.

As already stated, ring rot is formed early in storage. Usually the roots are not marketed until alfter about four to eight weeks, often much later, from the time they are stored. It is then that the disease attracts attention from the storage men and specimens are shipped in for identification. At this period, however, *Nectria ipomoeae*, *Trichoderma Köningi*, *Fusarium batatis* and several other fungi may constantly be found, separately or associated, invading the tissue of the dead ring from which the primary parasite has long disappeared. This year, in studying the diseases of

[17] Cook, Mel. T. and Taubenhaus, J. J. Trichoderma Koningi the cause of a disease of sweet potatoes. Phytopathology **1**: 184–189. 1911.

the sweet potato in storage, the ring rot was looked for as soon as the roots were stored. My recent studies have shown that the ring rot is caused by the fungus *Rhizopus nigricans*, which is also the cause of the soft rot of the sweet potato.

*Proofs that ring rot and soft rot are both caused by the fungus R. nigricans Ehr.* Ring rot is similar to soft rot except that in the former the decayed area is limited to a ring. The probable reasons for ring formation will be alluded to later. In our previous studies we failed to learn the cause of ring rot, simply because we worked on the dry ring stages. Ring rot, like soft rot, is formed as the roots are first stored and undergo the "sweat." At this stage there is not only a difference in resistance of the different roots to soft rot but there seem also to exist in the same root, zones of different resistance, the weaker zone alone yielding to the attacks of Rhizopus, and thereby limiting its activity to a ring-shaped area. Because of limited space to fully describe my studies on this disease, I will but state the more important conclusions reached.

1. Ring rot, like soft rot, starts early when the sweet potatoes are first brought into storage.

2. In fresh ring rot, as in fresh soft rot, the fungus does all the damage in a very short time, usually within twenty-four to forty-eight hours. The infected parts in both rots are very soft and water-soaked. In both, the water may leak out through some break in the epidermis, the tissue drying in proportion as the water is lost.

3. Like soft rot, ring rot, under storage conditions, does not send out sporophores unless a break occurs on the epidermis and this usually happens through the weight and pressure of the surrounding roots. In this case the sporophores are short, very numerous, closely packed, and borne at the break in the epidermis.

4. As in soft rot, when roots freshly infected with ring rot are placed in a moist chamber, the fungus *R. nigricans* grows out at the area of the ring within twenty-four hours (pl. XVIII, figs. 20–23)

5. In plating out tissue from sweet potatoes freshly infected with ring rot, a pure culture of the Rhizopus fungus is obtained in from twelve to sixteen hours.

6. Within a week to ten days after infection, the diseased tissue of ring rot, just as in soft rot, is odorless, but is soon followed by fermentation. At this stage the causative organism begins to die.

7. Ring-rotted sweet potatoes, like soft-rotted ones, after reaching an age of ten days to three weeks, upon being placed in a moist chamber, fail to develop any Rhizopus growth from the infected area. Platings made from these roots fail to produce any Rhizopus growth, but on the contrary produce fungi like *Oospora lactis*, *Oosporoideae lactis* and bacteria (pl. XVIII, figs. 24, 25 and 26). Crush mounts of the tissue from

old ringed areas under the microscope reveal the presence of the Rhizopus fungus, the filaments, however, being either empty or the protoplasm browned and broken up into small granules, indicating the absence of life in these hyphae.

8. Last, but not least, ring rot can be reproduced by inoculating fresh spores from a pure culture of *Rhizopus nigricans* into punctures made in healthy but susceptible sweet potato roots placed in a moist chamber and kept from 70 to 80° F. The fungus can readily be reisolated from the fresh soft ring, but if a week to ten days is allowed to elapse after the formation of the artificial ring, an active fermentation sets in and the caudal fungus soon dies out.

*Possible reasons for ring formation.* The question now arises, why does the fungus *Rhizopus nigricans*, when attacking sweet potatoes, turn some roots into a soft mushy rot and in other roots limit the injury to a ring? The reason is, no doubt, physiological rather than pathological, and some tentative suggestions may be given. The conditions favorable for soft rot are a susceptible individual sweet potato, an injury through which the fungus can penetrate (although this does not always seem necessary), a humid atmosphere and a fairly high temperature. Sweet potatoes which are most susceptible to soft rot are usually those which are large and well ripened. Most of these may be readily infected with Rhizopus and the typical soft rot reproduced at will. Such roots do not show a tendency to sprout, indicating a possible low enzymic content over-balanced by a higher per cent of sugar, and therefore more watery. Sweet potatoes which are covered with sprouts are very difficult, if not impossible, to infect with soft rot. Such roots, however, may usually be subject to ring rot, indicating a possession of resistant and susceptible areas in the same root. The formation of the ring rot seems, therefore, an accidental process where the fungus strikes the susceptible area Uneven ripening may also be a factor in ring rot formation. The susceptible area in this case probably contains a higher percentage of sugar, whereas the resistant areas may contain greater enzymic activity and more starch, apparently unfavorable to the fungus, thereby limiting its area to the weaker zone. Roots which long resist the attacks of Rhizopus, often suddenly lose their resistance and become a ready prey to the fungus. This is equally true for the ring rot where parts of the resistant areas suddenly become susceptible and a new ring is formed next to, or near, the old one. Sometimes the entire part next to a ring loses its resistance and turns into soft rot (pl. XVIII, figs. 20 and 21). We have met with this condition time and again while studying this disease. Considerable difficulty is experienced in reproducing the ring rot artificially, simply because it is difficult to locate the susceptible areas. In this case a large series of inoculations is necessary in order to strike upon the susceptible

area in the highly resistant root. In dealing with unsprouted roots, the chances are that they are non-resistant at the start and they will nearly all, if artificially inoculated, go to soft rot. Occasionally, however, a few are found among such roots which show partial resistance. In this case, the typical ring is easily reproduced, but it only lasts from five to six days as the fungus is seen to gradually advance until the entire root has gone to soft rot.

### VINE WILT OR YELLOWS (STEM ROT)

*Fusarium batatis* Woll.

In 1890, Halsted[18] was the first to call attention to a vine disease of the sweet potato which he termed stem rot, but, as we shall see later, this term is ill applied to the disease. The cause of the disease was not given. In 1891 Halsted[19] attributed the cause of the above disease to the fungus *Nectria ipomoeae* Hals. He also found a similar organism growing on egg plants and concluded that the fungi on the two hosts were the same. On both hosts he found a Fusarium constantly associated with the Nectria. My own work on this disease for the past three years conclusively shows that a species of Fusarium and not *Nectria ipomoeae* is the cause of the stem rot. We have been unable to find any Nectria stage produced by this Fusarium. *Nectria ipomoeae* always produces fusiform spores in pure culture, and either stage can be produced from the alternate stage.

Stevens,[20] in writing of a sweet potato wilt notes the following: "This disease destroys more than half of the crop on farms in many portions of the state." In 1907 he again[21] reports a new disease which he terms "soil rot." "We have found," he writes, "a fungus Fusarium which is constantly associated with the disease and which seems probably to be the cause of it." That this is the same disease with which we have been working, and the same as Halsted's stem rot, there is no doubt. It simply supports our conclusions and that of Harter[22] who has recently shown that *Fusarium batatis* Wr. and not *Nectria ipomoeae* Hals. is the cause of the

[18] Halsted, B. D. Some fungous diseases of the sweet potato. N. J. Agr. Expt. Sta. Bul. 76: 31–32. 1890.

[19] Halsted, B. D. The egg plant stem rot (Nectria ipomoeae Hals.). N. J.Agr. Expt. Sta. Ann. Rept. **1891**: 281–283.

[20] Stevens, F. L. Report of the Biological Division. (Sweet potato wilt.) N. C. Agr. Expt. Sta. Rept. **1905**: 20–29.

[21] Stevens, F. L. Report of the Biologist. N. C. Agr. Expt. Sta. **1907**: 15–19.

[22] Harter, L. L. and Field, Ethel C. Fusarium batatatis Woll. M. S., not Nectria ipomaeae Hals., the cause of the Sweet potato stem rot. Phytopathology **3**: 68. 1913.

Die Welkekrankheit oder Stengelfaüle der Süsskartoffel (Ipomoea batatos Poir.) Zeitschr. Pflanzenkrank. **24**: 204–207. 1914.

stem rot. In my own studies and observations I find that *Nectria ipomoeae* is a frequent secondary invader in almost any of the sweet potato diseases. It is often found following soft rot and particularly black rot. It also seems to be more prevalent in some years than in others In 1911 the fungus was very abundant on sweet potatoes decayed from various causes in Delaware. In 1912 the fungus was very scarce and in 1913–14 it has been fairly abundant again.

*Symptoms.* The symptoms which are given by the older writers are rather misleading. According to Halsted[23] "The line between the decayed and healthy portion is sharply drawn; in the former there only remains a blackened substance with the skin loosely attached." This, of course, leaves the impression of a rot being formed at the stem end, hence the term stem rot. Duggar,[24] too, in his description leaves the impression of a rot being produced by this disease as he writes: "The vine while young, turns yellow, then black near the ground and dies at or near the surface of the ground; but if rotted at some joint, it remains green beyond that point." With this disease, there is no rotting of the stem in the sense that the term rot is understood today, nor is there any rotting at the joints such as the above-quoted author describes. This is more characteristic of the black rot or black shank, *Sphaeronema fimbriatum* (E. & H.) Sacc. The term stem rot seems rather inappropriate, and stem wilt, vine wilt, or yellows is more applicable.[25] The symptoms of the disease are a yellowing of the stems and a slight yellowing and drooping of the leaves. Usually in a diseased hill there is more than one infected vine, and often every stem shows the disease. Infected stems are usually paler nearest to the stem end, although in this case the disease may extend to the tip. The disease causes no external rotting or blackening of the affected parts. Usually, too, infected plants will persist until digging time and even produce fair-sized roots, provided no secondary invader comes in (pl. XIX, figs. 29–32). However, the black rot is often a frequent follower of vine wilt. The former then adds the blackness to the affected stem which is mistaken for a symptom of the primary disease. Quite often the infection with the above secondary invader is confined to the base of the stem nearest the ground. In this case, entire death of all the vines of the hill results in a very short time (pl. XIX, fig. 34). The dying does not extend to the roots, as new shoots are sent out a week or two later (pl. XIX, fig. 33). Not infrequently a wet rot sets in at the

[23] Halsted, B. D. N. J. Agr. Exp. Sta. Bul. 76: 3–30. 1890.

[24] Duggar, J. F. Sweet potatoes, culture and use. U. S. Dept. Agric. Farmers Bul. 26: 5–29. 1895.

[25] Manns, T. F. and Taubenhaus, J. J. Some practices in sweet potato growing to avoid loss from disease. The Del. Farmer **1**: 5–9. 1914.

base of the plant where black rot has followed stem wilt. Here the entire plant may easily be pulled out. This stage has, no doubt, helped in causing the errors which our early workers made in describing "vine wilt." Yellowing of the stems and vines, while a constant symptom, is not always very pronounced. Often plants which are diseased can hardly be detected, because of the green color and the way the plant is thriving in spite of the disease. In these cases, roots are formed which look sound in every respect except that the fungus is hidden internally in the fibro-vascular bundles. Such plants, too, usually yield roots which come up to standard size of seed (pl. XIX, figs. 29 and 30). These, of course, help to carry over the fungus the following year. Infected stems, when split open, reveal a browning of the vascular bundles, indicating the presence of the parasite (pl. XIX, figs. 37 and 38). Vine wilt is more of a field disease than a storage trouble. Seeds which are infected may be carried over in storage with apparently no progress being made by the disease. In larger, infected roots the fungus makes more headway and produces a dry punky rot. Such roots have a peculiar cinnamon odor and a deep chocolate color and at the same time become light and shriveled. Wollenweber[26] has recently shown that two Fusaria are involved in the stem wilt disease of the sweet potato, viz.: *Fusarium batatis* Wr., and *Fusarium hyperoxysporum* Wr.

*Pathogenicity.* During my three years of study on this disease, *Nectria ipomoeae* could at no time be made to grow on healthy sweet potatoes. On the other hand, a Fusarium was constantly associated with the diseased roots. The fungus may readily be isolated by taking internal portions after surface sterilizations, yielding in nearly every case a pure culture of the parasite (pl. XIX, fig. 36). Pure cultures of the Fusarium have been inoculated several times on plants in the field, the fungus being inserted at the foot of the plant through a slight wound. Checks were also prepared. In from two to five weeks the typical vine wilt was reproduced. Healthy roots which had also been previously disinfected and kept in the laboratory were placed in moist chambers and inoculated with pure cultures of the fungus. The roots were clipped at both ends with a sterile knife to determine the soundness of the interior tissue and of the fibro-vascular bundles. The fungus was inserted with a sterile needle in slits made in the epidermis, or on both ends at a bundle. Typical punky rot was reproduced in seven weeks, showing the slow nature of the disease on the roots. Further work on this disease is now in progress. The results obtained will appear in a later publication.

[26] Wollenweber, H. W. The identification of Fusarium occuring on the sweet potato. Jour. Agr. Research., U. S. Dept. Agr., Washington, D. C. 1914.

A NEW LEAF SPOT

## Septoria bataticola n. sp.

A new leaf spot which attacks the foliage in the field has been observed for the first time by the writer. This disease is characterized by minute white spots ranging from one-eighth to one-third inch in diameter. These are chalky white, bordered by a brown area, and upon close observation are found to bear a few minute pycnidia. The white areas readily drop out. This leaf spot is found wide spread on sweet potato foliage in Delaware. The disease is attributed to what appears to be a new species of Septoria and is therefore named *Septoria bataticola* n. sp. A description of the fungus follows:

Pycnidia varying from one in the center to a few scattered ones, in spots which are mostly circular and sparsely or thickly cover the leaf, chalky white, bordered by a brown area. Pycnidia imbedded under the epidermis with, open mouths protruding or free, varying from 70 to 130$\mu$; readily falling out. Spores hyaline, vermiform, curved to straight, varying from 15–80 x $\frac{1}{3}$–$\frac{1}{2}\mu$. Parasitic on living leaves of sweet potato, *Ipomoea batata*.

Type material will be deposited in the herbarium of the Delaware Agricultural College Experiment Station, and at the National Museum at Washington, D. C.

DELAWARE COLLEGE AGRICULTURAL EXPERIMENT STATION
NEWARK, DELAWARE

EXPLANATION OF PLATE XVII

FIG. 1. Fruits of Rhizopus nigricans Ehr. on a freshly soft-rotted sweet potato placed in a moist chamber for twelve hours.

FIG. 2. Sweet potatoes artificially soft-rotted by smearing the roots with spores of R. nigricans and covering the chamber. In photographing, the cover was removed.

FIG. 3. Sweet potatoes treated as in figure 2, but the chamber left uncovered. Notice the healthy potatoes.

FIG. 4. Sweet potatoes soft-rotted by smearing the roots with spores of R. nigricans and covering with a bell jar without a drying agent.

FIG. 5. Same treatment as in figure 3, plus acid as a drying agent. Notice the healthy roots. The bell jars were removed while photographing figures 4 and 5.

FIG. 6. Healthy sweet potato.

FIG. 7. Soft-rotted sweet potato as it has naturally dried in storage without producing the spores of R. nigricans.

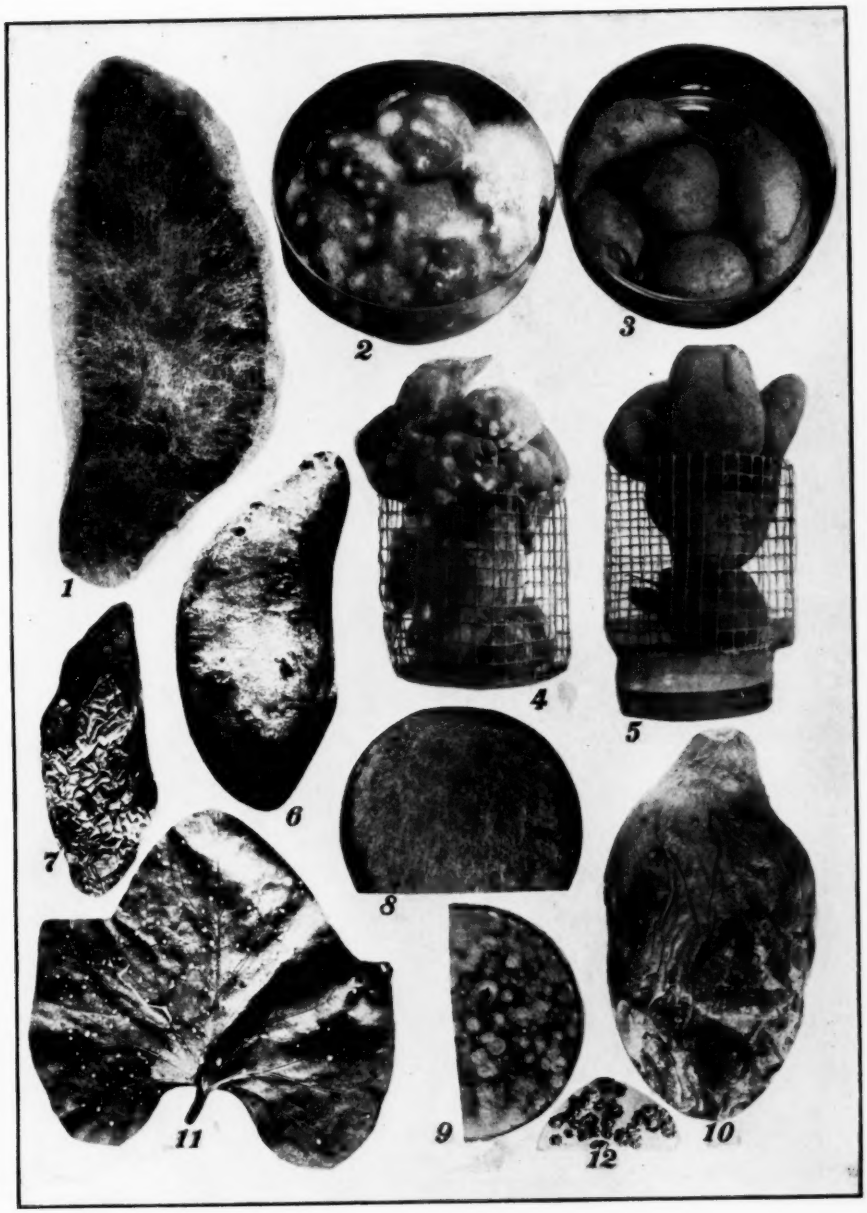
FIG. 8. Pure culture of R. nigricans from tissue of sweet potato freshly infected with soft rot.

FIG. 9. Culture of Oospora lactis, and Oosporoidea lactis, from tissue of soft-rotted sweet potatoes fifteen days old. R. nigricans has completely died out.

FIG. 10. Soft-rotted sweet potato in storage with breaks in the epidermis. Tufts of short-styled sporangia of R. nigricans as seen at the place of the cracks.

FIG. 11. Septoria leaf spot of sweet potato.

FIG. 12. Pure culture of Septoria bataticola.




TAUBENHAUS: DISEASES OF SWEET POTATOES

## Explanation of Plate XVIII

Figs. 13, 14, 16 and 18. Ring rot of the sweet potato, showing a single ring.

Fig. 15. Same, showing 2 rings.

Fig. 17. Same, showing 3 rings.

Fig. 19. Same, showing 4 rings.

Fig. 20. Freshly formed soft rot next to an old ring.

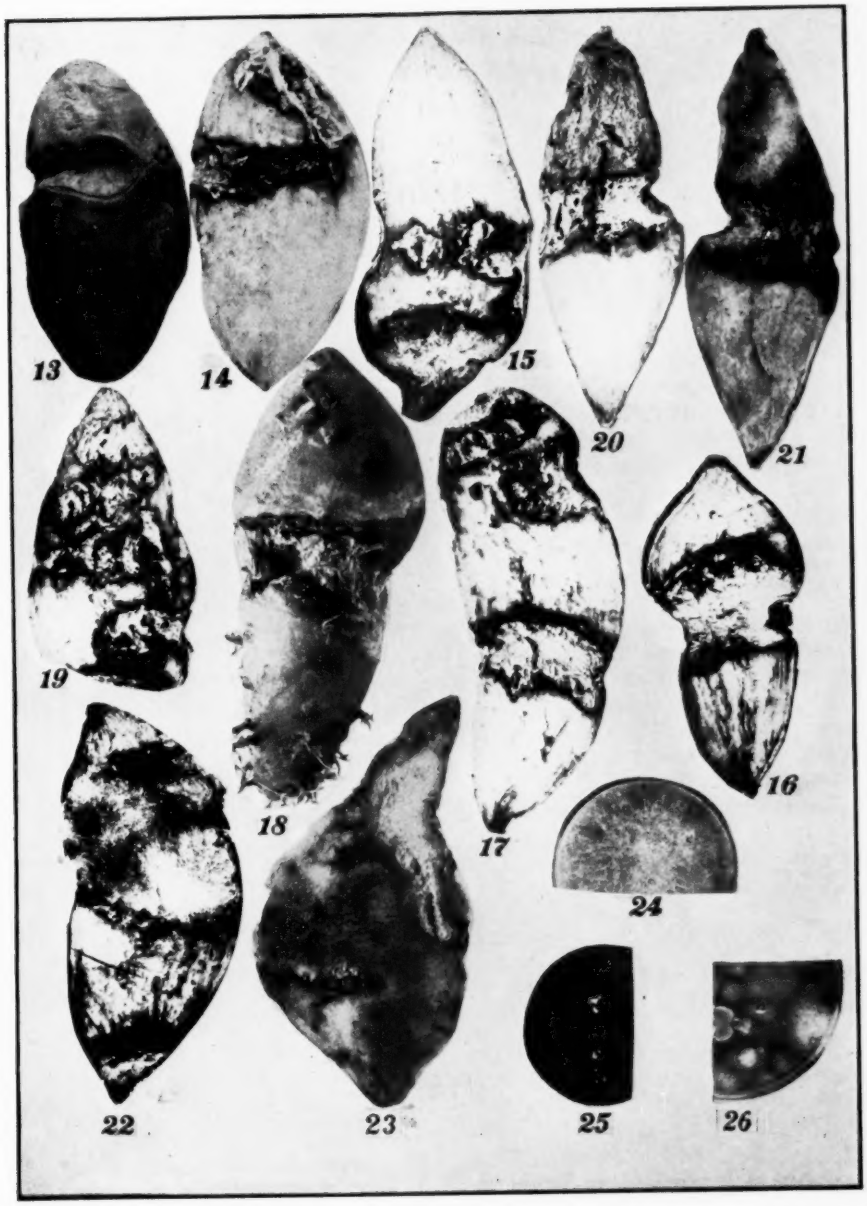
Fig. 21. The same placed in a moist chamber. Notice the total absence of fruits of R. nigricans on the old ring; this is confined to the freshly soft-rotted portion of the root.

Figs. 22–23. Ring rot of sweet potato two days old and placed in a moist chamber. Notice the fruits of R. nigricans on the areas of the fresh rings.

Fig. 24. Pure culture of R. nigricans from tissue of fresh ring rot after twelve hours.

Fig. 25. Culture, after forty-eight hours of Fusarium sp. bacteria and Oosperoidea lactis from tissue of old ring.

Fig. 26. Same as figure 25, after twelve hours. Note absence of R. nigricans.




TAUBENHAUS: DISEASES OF SWEET POTATOES

## Explanation of Plate XIX

Fig. 27. Roots from a hill affected with stem wilt, the stems of which have been killed by invaders other than Fusarium batatis Woll.

Fig. 28. Cross section of a root from figure 27 to show diseased fibrovascular bundles.

Fig. 29. Cross section of a root from figure 30, to show presence of disease.

Fig. 30. Roots from a hill affected with vine wilt, the stems of which have not been killed by secondary invaders.

Fig. 31. Roots from a healthy hill.

Fig. 32. Cross section of a root from figure 31, to show absence of disease.

Fig. 33. Hill infected with vine wilt, the stems of which were killed by secondary invaders. A new growth of vines is forming at the center of the plant.

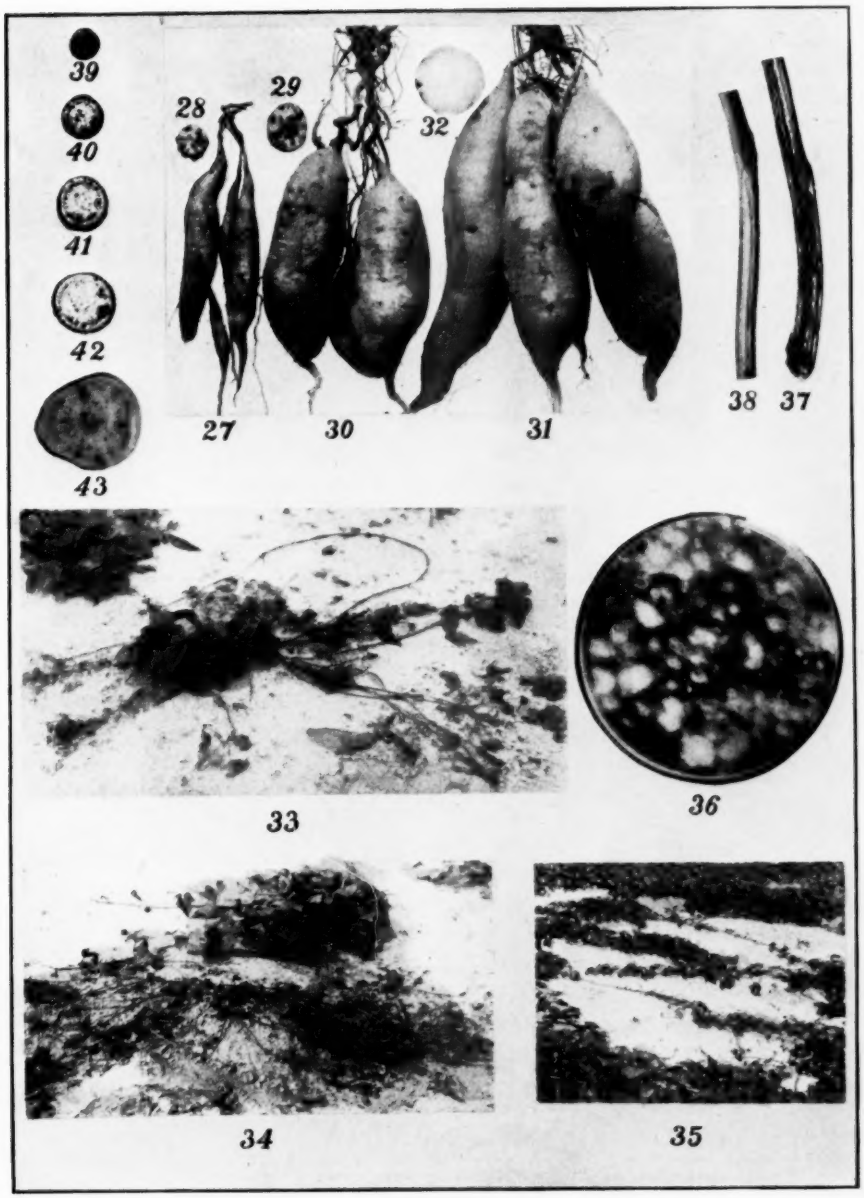
Figs. 34 and 35. Hills affected with vine wilt, the stems of which have been killed by secondary invaders.

Fig. 36. Pure cultures of Fusarium batatis Woll. obtained from the interior tissue of an affected vine.

Fig. 37. Stem of sweet potato affected with vine wilt. The epidermis was stripped to show the browned fibro-vascular bundles.

Fig. 38. Healthy vine of sweet potato.

Figs. 39–43. Cross sections of a root infected with Fusarium batatis Woll., showing diseased fibro-vascular bundles.




TAUBENHAUS: DISEASES OF SWEET POTATOES

# NOTES ON POTATO DISEASES FROM THE NORTHWEST

F. D. Bailey

With Plate XX

## SPONDYLOCLADIUM ATROVIRENS

On May 7, 1913, an inquiry concerning a disease of potatoes which was described as "of a queer nature" and which occurred on both early and late plantings on new ground was received from Albany, Oregon. This proved upon investigation to be silver scurf, *Spondylocladium atrovirens* Harz.

During the fall of 1913 this disease has been observed on potatoes from Clatsop, Columbia, Crook, Hood River, Linn, and Benton Counties and on the Station grounds it has appeared on potatoes of several varieties and from a number of sources. Spondylocladium has recently been reported from Western Washington by Rees.[1]

It is interesting to note that spores (30) developed on tubers in a moist chamber at room temperature in darkness and spores (30) developed on tubers in the field were 4–8 septate, 38.93 by 9.28$\mu$. These measurements agree with those for the species found in the eastern part of the United States by Clinton[2] and Melhus.[3]

## STYSANUS STEMONITIS

During the summer of 1913, a fungus was isolated from both stems and tubers of potatoes which proved to be *Stysanus stemonitis* (Persoon) Corda. In every case so far observed it was found developing in wounds or irregularities of the surface. It has been shown to be parasitic on tubers by Appel and Bruck[4] and was reported as the cause of a serious disease in Nebraska in 1901, by Bessey.[5] The writer knows of no further record of its occurrence in this country as a disease producing organism.

[1] Rees, H. L. Silver scurf disease. West. Wash. Exp. Sta. Mo. Bul. **1**: 15–16 F. 1914.

[2] Clinton, G. P. Report of the botanist. Conn. Agr. Expt. Sta. Rept., **1907-1908**: 357–359. 1908.

[3] Melhus, I. E. Silver scurf, a disease of the potato. U. S. Dept. Agr., B. P. I. Circ. 127: 15–25. May 1913.

[4] Appel und Bruck. Stysanus stemonitis (Pers.) Cda. und seine Rolle als Parasit der Kartoffel. Arb. Kais. Biol. Anst. f. Land u. Forstw., **5**: 441-448. 1907.

[5] Bessey, C. E. The brown disease of potatoes. Science **15**: 274. 1902.

ARMILLARIA MELLEA

In December, 1912, a case of what appeared to be mushroom root rot, *Armillaria mellea*, Vahl. on potato tubers was sent to this laboratory by a grower in Clark County, Washington. At that time a very limited number of specimens could be found and the several attempts at isolation, both from the decaying tuber and from rhizomorphs that radiate from the diseased areas, yielded either no organism or what were apparently secondary invaders. While proof is lacking to establish the identity of the organism causing the rot, an examination of the affected specimens and a comparison with the disease described by Johnston[6] indicates that the two are the same. The rhizomorphs were firmly attached at several points each of which was the center of an affected area. In the larger areas the white sheets of mycelium were found invading the tissue.

The ground on which the crop was raised was comparatively new and contained fir roots on which an abundant development of rhizomorphs was observed. It is further known[7] that this fungus has caused extensive losses in the prune orchards of Clark County.

The loss was of little consequence in the potato field and the grower was but little concerned.

In November, 1913 an inquiry was sent to this grower and he reported finding a few tubers affected with mushroom root rot in August and September. He was unable to furnish specimens, however.

No record of Armillaria rot of potato tubers in this country has come to the attention of the writer.

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[6] Johnston, T. H. Notes on a fungus found destroying potatoes. Agr. Gaz. N. S. W. **21**: 563–566 and 699–701. 1910.

[7] Piper, C. V. and Fletcher, S. W. Root diseases of fruit and other trees caused by toadstools. Wash. Exp. Sta. Bul. 59. 1903.

BAILEY: POTATO DISEASES

Rhizomorphs of Armillaria adhering to potato tuber. Note the rot spreading from the points of attachment.

# PRELIMINARY NOTES ON THE CULTIVATION OF THE PLANT PARASITIC NEMATODE, HETERODERA RADICICOLA

L. P. Byars

With Plate XXI

## INTRODUCTION

Two years ago, a study of the gall-producing nematode, *Heterodera radicicola* (Greef) Müller, was begun to determine, among other things, why certain plants are resistant to the parasite. In order to investigate under optimum conditions this intimate relation between host and parasite, it seemed highly important to evolve some pure culture method by which the various developmental stages in the life cycle of the latter could be observed continuously. After many unsuccessful attempts, such a method was found in March of the present year, and is outlined below.

## CULTURING THE ORGANISM

*The eggs.* Eggs, obtained from mature females growing within the root-galls of tomatoes or lettuce plants, were teased apart in a watch glass from the gelatinous substance which holds them aggregated in masses and treated either with mercuric chloride 1: 1000 for twenty minutes or with pure 3 per cent acid-free hydrogen peroxide for one-half hour. After the former treatment, the mercuric chloride was drawn off with a sterile pipette, and then the eggs, after they had been washed several times in sterile distilled water, were transferred in about 1 cc. of this liquid to a sterile petri dish. Eggs disinfected with hydrogen peroxide were transferred directly to the dishes without washing. Clear organic media such as Thaxter's potato hard agar or lima bean agar were at first used for pouring the plates; but later Pfeffer's nutrient agar, a synthetic medium, gave so much more satisfactory results that it was used exclusively. As later pointed out, this synthetic agar was also used for growing the host plants.

The Pfeffer agar used was made according to the following formula:

| | |
|---|---|
| Calcium nitrate ($CaNO_3$) | 4.0 grams |
| Potassium nitrate ($KNO_3$) | 1.0 gram |
| Magnesium sulphate ($MgSO_4$) | 1.0 gram |
| Potassium dihydrogen phosphate ($KH_2PO_4$) | 1.0 gram |
| Potassium chloride (KCl) | 0.5 gram |
| Ferric chloride ($FeCL_3$) | Trace |
| Distilled water | 6.0 liters |
| Powdered agar | 12.0 grams |

With the low power objective of compound microscope the eggs, about 88 by 35 microns, can be readily detected in the plate and their subsequent development observed. Within some of the plates which remained sterile, many active thread-like larvae, about 400 microns in length, were produced in from one to several days, depending mainly upon the age and development of the eggs used. Usually only a small percentage of the eggs hatched, but sometimes as many as 50 per cent produced larvae. Upon escaping from the egg covering, the larvae moved actively through the medium in any direction, occasionally, however, accumulating in numbers within a drop of water which had collected upon the surface of the medium.

After sterilization the eggs also hatched equally well when placed in a drop of the agar upon a sterile cover glass, which was then inverted over a hanging drop slide and sealed with vaseline. The hanging drop cultures have the advantage over the poured plate cultures in that the eggs can be observed under the high power objective and in that the subsequently hatched larvae can be easily transferred. It is difficult, however, to prevent bacterial contamination in making hanging drop cultures, and for this reason the poured plate method was largely used.

*The larvae.* Within a small portion of uncontaminated medium, a number of sterile larvae, obtained by either of the above methods, were transferred by the aid of a sterile platinum needle to a tube of Pfeffer's nutrient agar, in which a sterile seedling of cowpea or tomato was growing. The method of culturing the host plants will be given in another paragraph. Usually the transfer of larvae was made at about the time the main root of the seedling had attained a length of 1 to $1\frac{1}{2}$ inches and before secondary roots had begun to appear, although some inoculations were made after secondary or even tertiary roots had been formed (pl. XXI, fig. 1). In all cases the transferred larvae were placed upon or just below the surface of the medium, and in from six to twenty-four hours, depending somewhat upon the length of the roots, most of them had become aggregated near the root tips as shown in figure 2. Thus, it seemed very probable that the root tip possessed some chemotactic stimulus which attracted the organism. Larvae, penetrating the epidermal cells near the embryonic region of a cowpea root tip were observed under the low

power objective the first day after their inoculation into the tube and by the end of the second day a macroscopic swelling of the root appeared some distance from its tip (fig. 3a). Most of the organisms remaining outside the roots died within a few days, although some continued active for more than a month. These active larvae did not, however, undergo their normal development into adults.

Upon entering the root tips, the larvae usually assumed a position within the cortex near the central cylinder, with their bodies at a slight angle or parallel to the axis of the root, and with their anterior ends pointing away from the root cap. The actual entrance of the nematode was not observed at other parts of the root than the tip, but that they had entered at other places—as at the base or secondary roots (figs. 3c and 4c) —later became evident when the enlarged tissue containing the parasites could be plainly seen with the unaided eye. Very often the secondary roots ceased elongating after the larvae had entered them, and instead their tips became much swollen (figs. 3a and 4a).

*The adults.* Once within the host tissue the parasites became motionless, increased greatly in thickness, underwent metamorphoses, and finally reached maturity. The mature females, flask to pear shaped, and measuring about 0.5 mm. in their greatest diameters, either remained buried within the host tissue as seen in figure 5a or ruptured it so that their posterior ends were exposed to the medium. Egg-laying females were found thirty-one days after the parasites had penetrated the root, and were observed to extrude their eggs at an average rate of approximately one each hour. Under the influence of strong artificial light, as while being examined under the microscope, egg-laying progressed more rapidly, sometimes at the rate of one in fifty minutes. Prior to and along with the deposition of eggs, the females also extruded a light brown, ribbon-like, gelatinous substance, which usually remained loosely attached to the posterior portion of the mature organism and in which the eggs were held massed together (fig. 5b). Some of these eggs were subsequently seen to hatch into active larvae. Thus, the life cycle of the parasite, from an egg of one generation to the egg of another generation was completed in artificial culture. The developing and finally mature males, a little more than 1 mm. in length and about one-fortieth of this measurement in thickness, were seen to escape from the enlarged roots and move about freely in the medium, but they were never observed to copulate with the females. That copulation does take place as suggested by Dr. Bessey[1] and other investigators, seems possible since females which never produced any eggs were frequently noticed in the cultures. However, it has not yet

[1] Bessey, Ernst A. Root-knot and its control. U. S. Dept. Agric., Bur. Pl. Ind. Bul. 217. 1911.

been determined whether fertilization by the male is necessary for the organism to complete its life cycle. A few days after escaping from the roots into the medium, the males became quiescent and soon died.

### CULTURING THE HOST PLANTS

The following method was used to secure sterile tomato or cowpea seedlings for inoculation purposes. Selected seeds were treated with mercuric chloride 1:1000, for fifteen minutes, washed in sterile water, and germinated in Thaxter's potato hard agar or Pfeffer's nutrient agar in petri dishes. The seeds were entirely covered with the medium and placed far enough apart in the dish so that those showing no contamination could be easily removed. Such seeds were trasferred to an ordinary test tube, half filled with Pfeffer's agar, usually when their hypocotyls were about one-quarter of an inch long. After the seedlings had grown for a day or more and there was no contamination evident, larvae of *Heterodera radicicola* from sterile plates or drop cultures were introduced into the tubes in the manner already described. Except when being examined, the roots of the host were kept darkened by pushing the lower half of the tube into pots of sand.

By preventing rapid growth of the seedling so that the plugs did not have to be removed from the tubes, plants were kept sterile for more than a month. Usually, however, the cotton plugs were either removed at the end of two weeks, or were later pushed out by the growing plant. By adding sterile nutrient solution from time to time, such exposed cultures have been kept growing for nearly three months.

### DISCUSSION

While Metcalf[2] obtained pure cultures of a nematode associated with plant decay, and Oliver[3] later grew under sterile conditions an ecto-parasitic animal species, the writer has seen no previous account in the literature where pure culture methods have been used in the cultivation of plant parasitic nematodes. It seems quite likely that some means similar to that outlined in this note might be used in the cultivation of other parasitic nematodes and particularly the destructive form, *Heterodera schachtii* Schmidt, which attacks beets. Similar methods may be found helpful in studying root parasites other than nematodes.

DEPARTMENT OF PLANT PATHOLOGY
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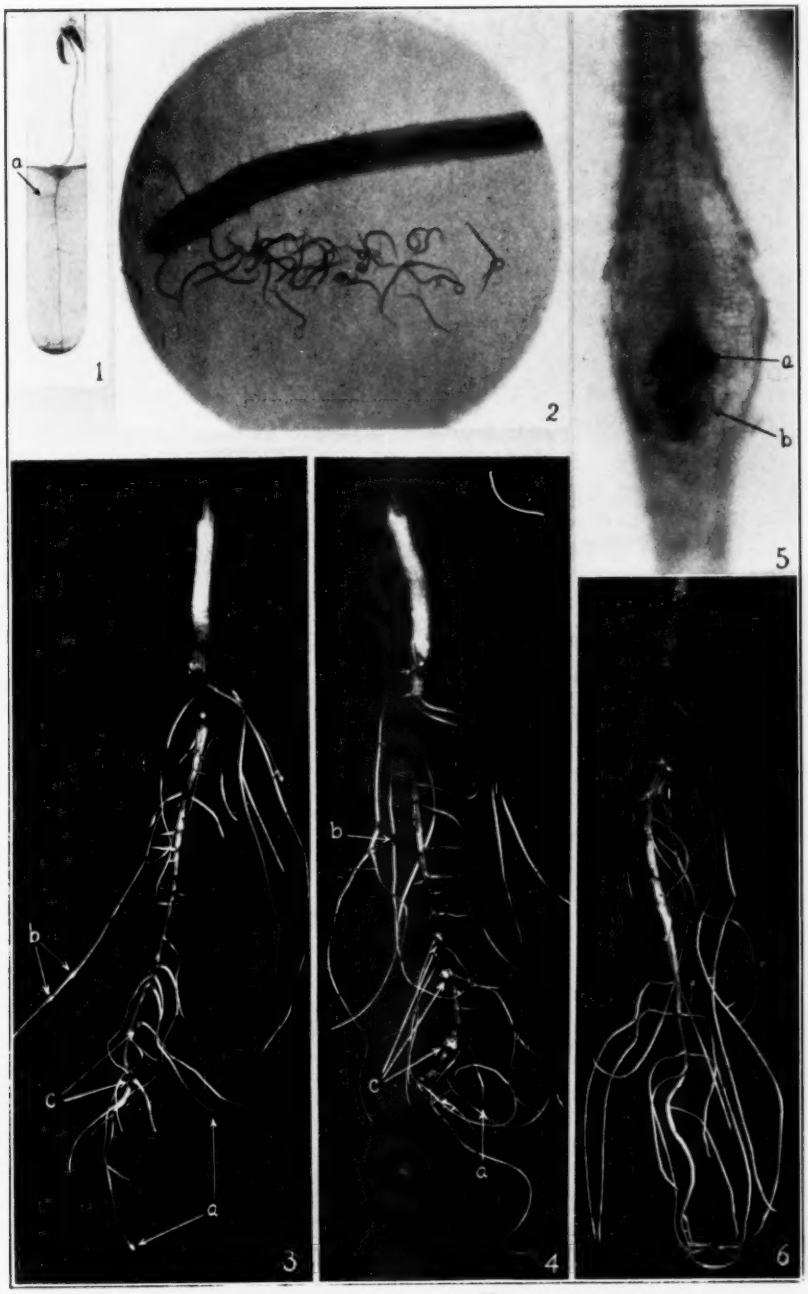


BYARS: CULTIVATION OF NEMATODE

## Explanation of Plate XXI

Fig. 1. Tomato seedling as grown in a tube of Pfeffer's nutrient agar. Larvae of Heterodera radicicola were placed in the medium several days before the photograph was taken and were seen aggregated near the root-tips as shown in figure 2. $\times \frac{1}{2}$.

Fig. 2. Photomicrograph of tip of secondary root marked *a* in figure 1, with active larvae aggregated about it. $\times$ 25.

Figs. 3 and 4. Root systems of cowpea seedlings grown in tubes of Pfeffer's nutrient agar and artificially inoculated with nematode larvae. The galls show various types of infection; *a*, ellipsoidal enlargements at tips of secondary roots; *b*, fusiform swellings some distance back from the tips; *c*, irregular galls at the base of secondary roots. $\times$ 1.

Fig. 5. Photomicrograph of fusiform swelling on secondary root of cowpea seedling, showing egg-laying female at *a* and extruded egg mass at *b*. $\times$ 25.

Fig. 6. Normal uninfected root system of cowpea seedling grown under the same conditions as those shown in figures 3 and 4 except for the absence of the nematodes. $\times$ 1.

# A DISEASE INVOLVING THE DROPPING OF COTTON BOLLS

J. Lee Hewitt

With Plate XXII and Two Figures in the Text

During the latter half of the summer of 1913 there was much concern in parts of Arkansas over the dropping of partly grown cotton bolls. The trouble was given a considerable airing in the newspapers and many theories were propounded and worn out in an attempt to explain the difficulty. The writer was in Little Rock during the latter part of August and took occasion to visit several cotton fields and to inspect many samples of injured plants, to see if, perchance, the nature of the trouble could be found.

The characteristic marks of the injury are as follows: The stem of a young boll, or sometimes of an older one or of a blossom, becomes paler in color and shrinks somewhat with slight lengthwise wrinkling of the bark, and apparently becomes much weakened. If the boll is large the stem may give way near the middle, permitting the boll to droop. Very soon the stem dries and the boll dies. It remains hanging on the plant for some time, however, and at the same time the disease extends downward along the fruiting stem of the plant killing the tissue on one side of it for a distance of $\frac{1}{2}$ to 2 inches. After the boll has completely dried, it falls away, taking with it this killed portion of the fruiting branch. This leaves a characteristic sign of the disease, as if the boll had been torn away while alive along with a splinter from the fruiting branch. This injury is distinctly different from the ordinary dry weather shedding of bolls. The writer found on this same trip some cotton patches which were suffering severely from drouth and examined the dropping bolls in these patches. In this case the bolls turn pale or yellow, the involucre or square being quite conspicuous by its yellowness. The bolls die and drop off before they dry up, and in dropping they leave a clean round scar with a corky layer over it.

This injury was wide-spread over the state, being perhaps most marked in the bottom land districts, where in some cases more than one-half of the bolls and forms which had set since July had fallen off by the last of August. The older bolls, that is those set from early blossoms, instead

of dropping off, opened prematurely. From this most severe injury there were all degrees down to 3 or 4 per cent of the entire crop.

Some specimens were gathered and taken to the office of Mr. C. W. Watson, State Demonstration Agent, at Little Rock, where a hasty examination was made of them in the hope of finding some parasite. No insect was found, and no indications of insect injury, on nearly all of the bolls. In a few cases boll worm, and in one or two cases boll weevil, were noted. A microscope was borrowed through the courtesy of the State Board of Health officers, and mounts in weak potash were made of scrapings from the pedicels of a number of injured bolls, hoping to find some fungous spores. No spores were found in great abundance, but there were constantly found from three or four up to fifty or more spores of an Alternaria-like character. An effort was also made at this time to find these same spores on the healthy portions of the stems and leaves. A few were found on the leaves and in only two cases were any found on healthy stems. The total number of examinations made was not exactly recorded, but, from a memorandum made three days later, it was estimated as follows: Examinations of diseased boll pedicels 50, examinations of leaves 3, and of healthy stems 8 or 10.

After returning to Fayetteville, the writer had samples sent to him from a cotton farm belonging to Mr. George Pye, County Demonstration Agent, at Mabelvale, south of Little Rock. Owing to pressure of work, the examination was delayed until December and January. In the meantime most of the samples became badly infected with a mold (*Cephalothecium roseum*) which made the examination of them difficult. In each examination an area 2 mm. square which had been moistened with a potash solution, was scraped clean and the scrapings were mounted on a slide for examination.

Following is a table showing the results of these examinations in searching for the Alternaria-like spores.

Laboratory No. 274. Only 1 spore was found on this plant on the involucre of a diseased boll. No other spores were constantly found. Some of the leaves of the plant showed the same characteristic injury as the bolls.

Laboratory No. 286. There were found altogether 30 Alternaria-like spores on this plant, distributed as shown in the table. The plant had 14 badly diseased bolls, 1 slightly diseased, 1 mildewed, probably with Cephalothecium, 1 injured by boll worm, and 3 healthy ones.

Laboratory No. 289. Only 3 spores were found in this case. Record was not made of the extent of the injury.

Laboratory No. 290. The leaves had been removed from this plant before sending it. It showed in all 16 spores, 12 of them being on the

Fractions express: $\frac{\text{Number of spores found}}{\text{Number of examinations}}$

| | LABORATORY NUMBER | | | | | | TOTAL ON DISEASED PORTIONS | TOTAL ON HEALTHY PORTIONS |
|---|---|---|---|---|---|---|---|---|
| | 274 | 286 | 289 | 290 | 292 | 302 | | |
| Outside of boll, diseased | 0/1 | 3/1 | 0/3 | 1/3 | 2/1 | | 6/9 | |
| Husk or square, diseased | 1/1 | 8/1 | 1/2 | 0/2 | 4/1 | 0/1 | 14/8 | |
| Husk or involucre of blossom, diseased | | | | *12/1 | | | 12/1 | |
| Pedicel of boll, diseased | 0/1 | 2/1 | 0/1 | 2/1 | 1/1 | 1/2 | 6/7 | |
| Petiole of leaf, diseased | 0/1 | 5/1 | 0/1 | | 6/1 | | 11/4 | |
| Branch, diseased area | 0/1 | 8/1 | 0/1 | 0/1 | 30/1 | | 38/5 | |
| Branch, healthy area | | 1/1 | | | †10/1 | | | 11/2 |
| Main stalk, diseased area at bases of leaves | 0/1 | 3/1 | | | | | 3/2 | |
| Main stalk, healthy area | | | 1/1 | 1/1 | 3/1 | | | 5/3 |
| Total | 1/6 | 30/7 | 2/9 | 16/9 | 56/7 | 1/3 | 90/37 | 16/5 |

* Mycelium in tissue

† Next adjacent to diseased area.

involucre of a blossom. In the tissue of this involucre was found the mycelium illustrated.

Laboratory No. 292. This plant had a total of 31 bolls, 6 of which were healthy, 13 plainly diseased, and 12 apparently diseased but obscured by the Cephalothecium.

After finishing with the examinations, an injured specimen was carefully scraped and washed in 20 cc. of physiological salt solution. The washing was divided into four parts, and centrifuged for two minutes. The sediment was carefully removed and found to contain a large number

of the Alternaria-like spores. Portions from it were placed in four petri dishes and covered with melted bouillon agar. A fifth portion was mounted on slides for examination with a microscope. On incubation all the plates failed to grow. Possibly this was because the spores were dead from long exposure, possibly because the melted agar, although care was taken to have it as cool as possible, may have been too warm.

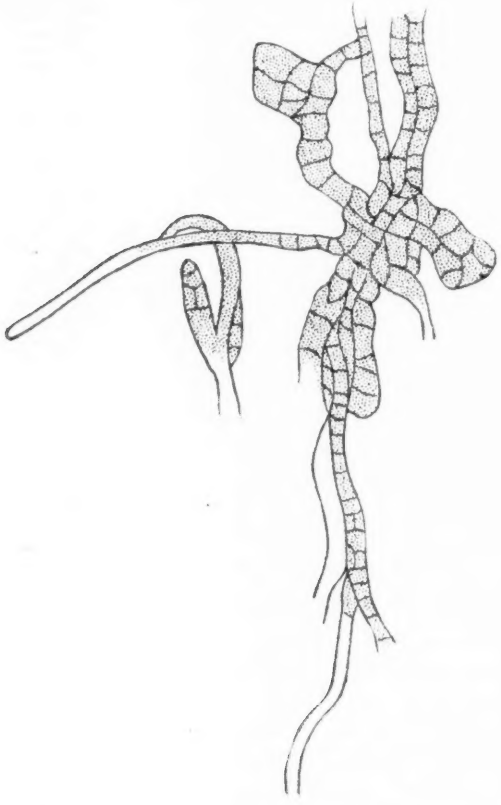

FIG. 1. Mycelium found in tissue of Laboratory 290.

Laboratory No. 302. This plant was so badly moulded as to give very unsatisfactory results: 1 spore was found; 3 bolls were healthy, 8 were diseased, 6 were badly molded, but probably not diseased with this trouble; 24 bolls were so badly molded that they could not be satisfactorily examined.

On August 22, the writer accompanied by Mr. Pye, the County Demonstration Agent, visited several farms to observe this trouble in the field.

At farm No. 1, which is rolling hill land and rather sandy, we found the variety Lowe's Mississippi badly injured, probably half of the bolls and forms were dropping off. The older bolls were opening even at this early date.

At farm No. 2, which is a rich sandy loam pocket between hills, we found the varieties Christopher Big Boll and Rowden somewhat infected, but the injury was not very great.

At farms Nos. 3, 4, and 5, slightly rolling valley farms in a region which had suffered severely from drouth, we found a little of this trouble and much of the characteristic dry weather injury. On No. 3, the variety was Lone Star, on the other two the varieties were not determined. On No.

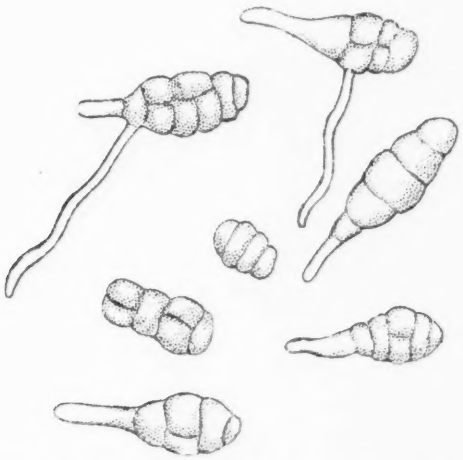

FIG. 2. Spores found on various injured portions of plants. The prevailing type large and many celled.

4 the cultivation had been poor and the dropping of bolls due to dry weather amounted to more than 10 per cent.

At farm No. 6, which is flat valley land, we found this disease in a considerable amount. There was no suffering from dry weather, and the plants were in good condition. We found also about 5 per cent of injury from boll worm.

At farm No. 7, which is bottom land, rolling somewhat, we found the variety Christopher Big Boll badly affected with this disease. This is particularly interesting because the cotton had not suffered at all for want of moisture, but was more than head high and very heavy and rank. There was a bad infestation of grass worm just beginning to show, but it had not caused any damage.

There were also visited eight more farms giving about the same results, more or less injury on each, and apparently no relation between the injury and drouth. In returning to Little Rock, ten or eleven more farms were observed from the buggy and the trouble was seen in all of them. On this trip there were also observed three cases in which cotton wilt was very severe.

From these things it will be seen that not enough has been done to determine the cause of the injury. The only indication of a cause has been the finding of Alternaria-like spores pretty constantly associated with it and the finding in one case on Laboratory No. 290 a small, dark, much branched mycelium in the tissue of an involucre.

The general appearance of the trouble would indicate that it is caused by some organism, possibly a fungus, having but a restricted growth, and possibly sterile, which may perhaps have gained entrance in the axil of the boll pedicel. The Alternaria-like spores may or may not be associated with the trouble.

In talking with many growers the idea seemed prevalent that this disease is present every year, but that only occasionally is it so severe as to cause appreciable injury. Some observant growers were inclined to attribute its prevalence to alternating wet and dry weather. The summer had been dry with light rains followed by rapid drying and hot wind.

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EXPLANATION OF PLATE XXII

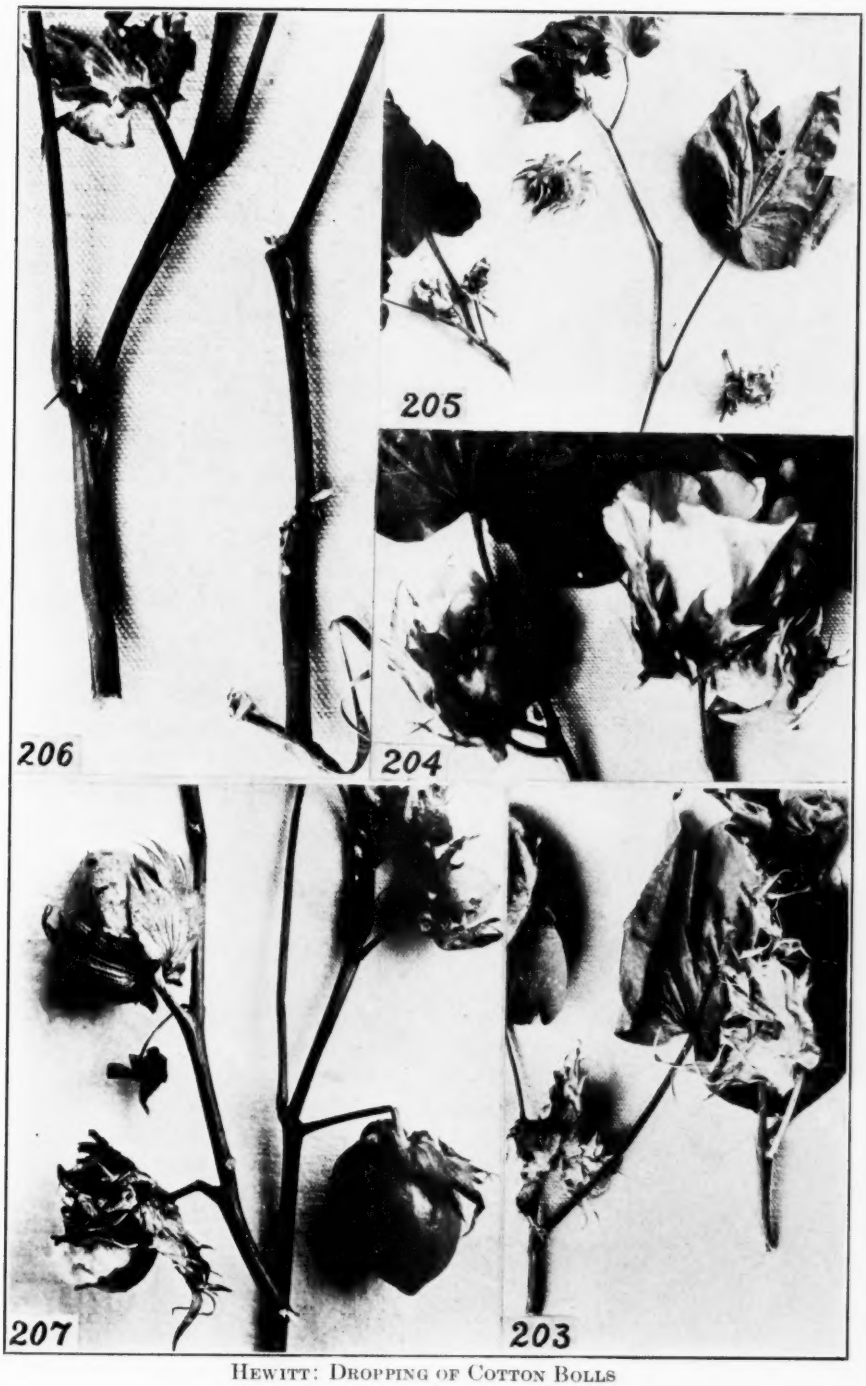
FIG. 203. Injury on squares and forms.

FIG. 204. Injured blossoms.

FIG. 205. Injury caused by the disease at left, contrasted with dry weather dropping at right.

FIG. 206. Injury, showing plainly the dead splinter.

FIG. 207. Injury to larger bolls, showing weakening of pedicel.




HEWITT: DROPPING OF COTTON BOLLS

# A FRUIT SPOT OF THE WEALTHY APPLE

E. C. Stakman and R. C. Rose

With Plate XXIII

During the fall and early winter of 1911 an unsightly spot of Wealthy apples was very prevalent in Minnesota. In 1912 it was not so widespread, but in 1913 it again assumed very considerable importance. The spots appear either in the field or in storage. Only ripe apples seem to be affected in the field, especially those which have been left too long and are slightly over-ripe. In cellars the spots may appear within a short time after storage, in some cases as many as 60 per cent becoming quite badly affected. The spots may also appear very promptly after apples are removed from cold storage.

The appearance of the spots is quite characteristic. The diameter varies from 2 or 3 mm. to 1 cm. or more. The color is brown or black; in the larger spots it is often a deep black. The spots are usually quite distinctly sunken, have very sharp borders, and are often around a lenticel. They are superficial, the dry brown tissue seldom being more than one or two millimeters deep. The spots enlarge only very slowly after once formed, and, although rotting may follow, it is frequently caused by other fungi.

Although the Wealthy is most frequently affected and the spot is called the Wealthy spot by Minnesota growers, it is by no means confined to this variety. It is very prevalent on Wolf River and Peter, both of which may be as severely affected as the Wealthy. It occurs also on Peerless, Red Mirror, Hibernal, and Patten's Greening. The opinion of growers is that the thin skinned varieties are most frequently and seriously affected.

Careful microscopic examination was made of spots from different varieties. In 70 per cent of all spots examined, including both small and large ones, very abundant mycelium of what later proved to be Alternaria was found. The mycelium was present in the entire diseased area and was sufficient in amount to account for the development of the spots. However, no mycelium was found in some cases. The possibility of two kinds of spots is not excluded, although macroscopic differences are not sharp.

Numerous attempts were made to isolate fungi. The surfaces of the spots were sterilized with mercury bichloride or iodine, washed with

sterile distilled water, and pieces of diseased tissue were then cut out with a sterilized scalpel. Checks were made from normal apple tissue. The pieces were placed on beerwort agar in petri dishes or on slants in test tubes. Alternaria developed with considerable consistency from the diseased tissue. Numerous isolations were made in 1911 and 1913, two persons working independently in 1913 with practically identical results. That the surface sterilization was effective is indicated by the fact that the checks produced no Alternaria.

Inoculations were made on Wealthy, Jonathan, and Ben Davis apples. No infection resulted from inoculations made without injuring the apples. In some cases agar was placed on the unbroken skin and the fungus allowed to grow on the surface. Even under the most favorable conditions, however, no infection resulted. When needle punctures were made the results were very different. Typical spots, in no way distinguishable from those naturally occurring on apples, developed. The spots were very distinct seven days after inoculation and in two weeks were in every way typical. The best results were obtained on Wealthy and Ben Davis apples. Typical spots developed on Jonathan apples also, but many of them were rather small. The size of spots increased rather slowly after two weeks. Wealthy apples were kept fifty-three days after inoculation, and, at the end of that time, the spots were from 6 to 11 mm. in diameter and confined sharply to the inoculated areas. Only one spot developed spontaneously in this time, no secondary rots had started, and there was no sign of spotting or rotting on the check apples which of course had been punctured in the same way as those which were inoculated. Ben Davis apples were kept forty-eight days after inoculation with exactly the same results (pl. XXIII).

Whether or not the spot is the same as the Jonathan spot cannot be stated with certainty, although the appearance of the two is very similar. Jonathan apples are not grown on a commercial scale in Minnesota, and the only apples of this variety available for comparison were those on the Twin City market. These were all western grown. Typical spots were very rare, although many small, dark, superficial spots were found on both the Jonathan and the Gano. These were not distinctly sunken and were in other features different from the typical Wealthy spot. From descriptions, however, it would seem that the typical Jonathan spot and the Wealthy spot are quite similar or possibly identical.

There is still some difference of opinion as to the cause of the Jonathan spot. Scott and Roberts (1) are of the opinion that it is physiological while Cook and Martin (2, 3) cite evidence which leads them to incline to the view that it is caused by an Alternaria which they have consistently isolated from diseased spots. Norton (4) has shown that typical

spots may be produced by the action of gases and suggests the possibility of this cause in the case of apples kept in cold storage. The primary cause of the disease in Minnesota is still somewhat a matter of doubt. The Alternaria which occurs in a large percentage of natural spots is capable of producing absolutely typical spots when introduced through needle punctures, but another season's work will be necessary before absolutely definite conclusions can be reached.

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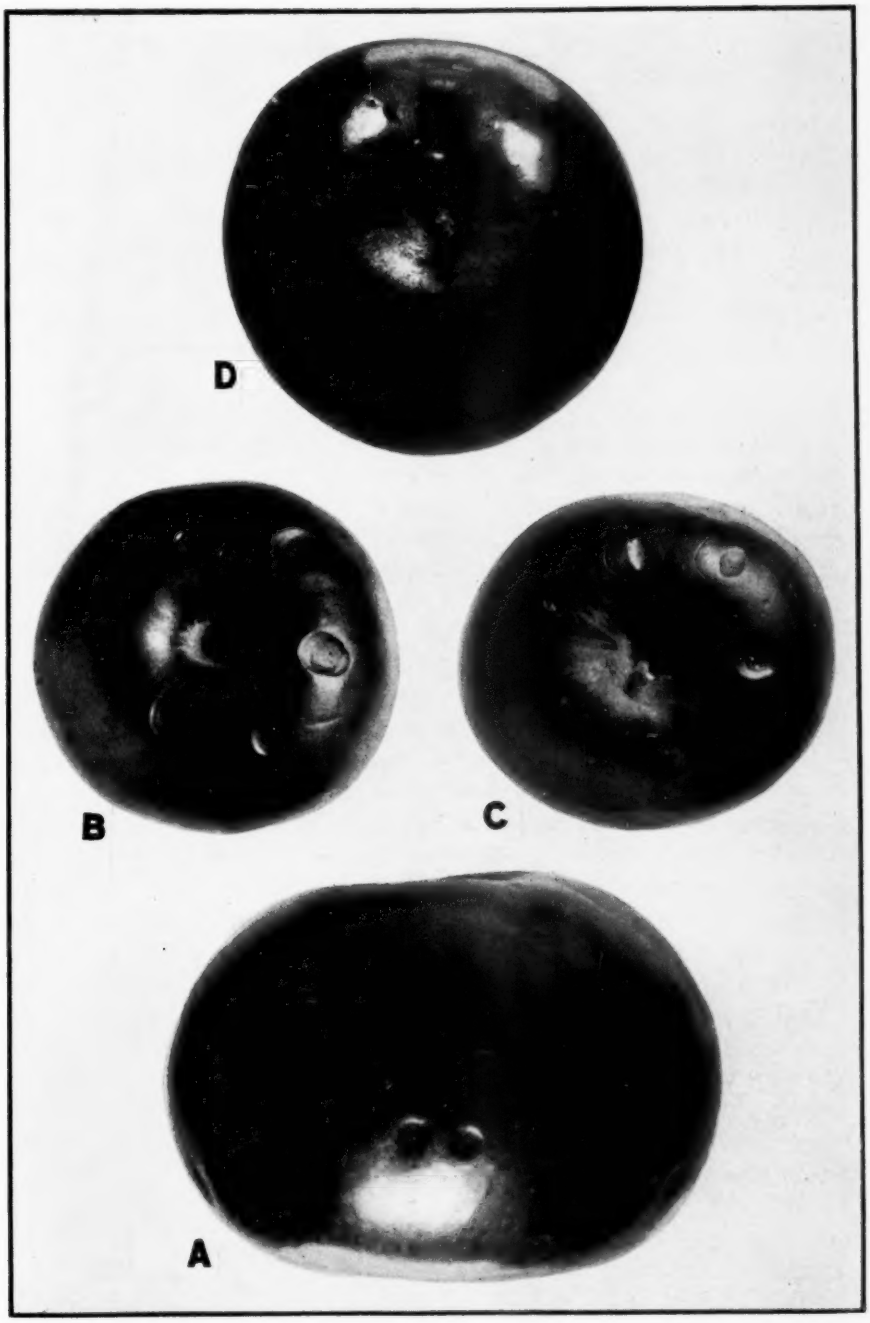
EXPLANATION OF PLATE XXIII

*A*, Wolf River apple showing naturally occurring spots.

*B* and *C*, Ben Davis apples showing spots developed as a result of needle puncture inoculations.

*D*, Wealthy apple. Check punctures showing inside of inked circle. Fifty-three days after puncturing.




STAKMAN AND ROSE: FRUIT SPOT OF WEALTHY

## PHYTOPATHOLOGICAL NOTES

*Wintering of timothy rust in Wisconsin.* In an article entitled "Investigations of Timothy Rust in North Dakota," in the February number of the current volume of PHYTOPATHOLOGY, W. H Mercer states that, under North Dakota conditions, the uredospores are very difficult to find after the first hard freeze and that he was unable to get them to germinate after October.

These results do not agree with observations made by the writer during the past winter at Madison, Wisconsin. Good germination was secured from uredospores collected in the field in October, November, and December. A 10 per cent germination was obtained from spores collected in the field January 18, and a smaller per cent, but with more vigorous germ tubes, was secured March 15. Spores were germinated in tap water on slides in a damp chamber and it was found that germination was much slower during the winter months. No difficulty was experienced in finding spores on green leaves in the center of clumps of grass along fences in places where the grass had not been cut. On March 20 several plants were taken up, potted, and kept in the greenhouse. They were placed under bell jars for several days to facilitate the development of overwintering uredospores. Fully developed sori were found April 13 on new leaves which had grown up around the old diseased ones, and also, on the same date, some of the old leaves which had wintered showed pustules developing on the old flecked places. It is my judgment that these later sori developed from the overwintering mycelium.

From the above results I must conclude that in this locality the uredospores of timothy rust can live over winter and infect the new growth in the spring, and also that the mycelium probably lives over winter as far north as Madison, Wisconsin. Data furnished by the local weather bureau office seem to indicate that the winter has been very unfavorable to the wintering of spores of any kind. The ground has been bare during most of the winter, the snowfall for every month being below the average; and, although the mean temperatures for the months of December and January were slightly higher than the average, the mean temperature for February was lower than it has been for ten years.

In general, the above results and conclusions seem to agree with those secured by E. C. Johnson at Washington, D. C., and reported in Bulletin No. 224 Bureau of Plant Industry, 1911.

The teleutospores of the timothy rust are not very plentiful in this locality, and I have found them only in shaded places, along fences or the edges of woods, never in the open. Dr. J. J. Davis, at whose suggestion I made these observations, tells me that the teleutospores are produced in abundance in the northern part of Wisconsin, rather early in the season, but always in shaded places.

CHAS. W. HUNGERFORD

*Peronospora parasitica on Arabis laevigata.* On April 25 of this year a single plant of *Arabis laevigata* infected with a Peronospora was found near Crawfordsville, Indiana. A few days later specimens were found in various localities about this city and a further search revealed the fungus in a number of widely separated areas so that it is evidently generally distributed in this region. Method of production, size, and form of the conidia together with the fact that the fungus occurred on a cruciferous host indicated that it was *Peronospora parasitica*. A specimen was sent to Dr. Guy West Wilson at New Brunswick who identified it positively and further stated that so far as he knew it had never before been reported on this species of Arabis. At his suggestion this note is published in order that the occurrence of *P. parasitica* on *Arabis laevigata* might be recorded.

The under side of the radical and stem leaves were infected at this time (the first of May), but in no case was there found a general infection of the whole plant. The infected plants were not dwarfed nor were the leaves markedly different from the uninfected ones. On this account it was often difficult to distinguish the diseased plants, and this might account for the fact that, in the past, it has generally escaped the notice of collectors. A careful search was made for oospores without success although old specimens have not yet been examined. *Peronospora parasitica* on *Dentaria laciniata* is very abundant in the regions where the infected Arabis plants occur, and cross inoculation experiments would no doubt be of interest.

Specimens of the infected plants are deposited in the herbaria of the Department of Plant Pathology, Cornell University, Missouri Botanical Garden, New York Botanical Garden and in the private collections of Dr. Wilson and the writer. No doubt a search of regions where this host occurs will reveal a much wider distribution of the fungus than is here indicated.

H. W. ANDERSON

*An unusual host of Fomes fomentarius* Fries A very interesting case of the occurrence of this fungus on various varieties of apple tree recently came to the writer's attention in an old abandoned orchard near Missoula, Montana. The trees were at one time badly pruned leaving great unhealed scars. Practically all the wounds on three different trees were infected and a few supported fruiting structures. The heartwood of the main trunk and of the larger branches in all the infected trees was entirely decayed. The mycelium in a number of cases in penetrating the sap-wood had caused a pronounced channel to form above and below the sporophores. Of all the varieties of apples in the orchard the Red McIntosh was most severely attacked. Some varieties (Jonathan) seemed entirely immune although badly wounded and in close proximity to trees bearing sporophores. This may either be due to an accident of spore distribution or that the wood of some varieties of apples is less susceptible to attack. The vitality of the infected trees was greatly reduced, and they had long since ceased to bear fruit. The diagnosis of two dead trees showed plainly that their death resulted from the activities of this fungus. In the West *Fomes fomentarius* chiefly occurs in the forest and on Betula, Salix, and Populus. This is the first notice of its occurrence in this region outside the forest and on fruit trees.

*Fomes applanatus* Persoon or *F. leucophaeus* Mont. as the American form is designated by some authors is more common in the West on cultivated fruit trees. Two collections are at hand from Sand Point, Idaho, both taken from the Spitzenburg variety of apple.

JAMES R. WEIR

*The cankers of Plowrightia morbosa in their relation to other fungi.* In a recent number of Phytopathology (**3**, p. 311), Professor Macbride notes the occurrence of *Fomes igniarius* (L.) Gillet on the wild plum infected by *Plowrightia morbosa* Sacc. Throughout Montana the cankers formed by this parasite on Prunus and Amelanchier are very frequently infected by the Fomes. This additional irritation causes the formation of large burls of very irregular shape and of a peculiar structure. The white rot of the Fomes is thrown in strong relief by the dark gum-infiltrated zones of the burl into which the mycelium of the fungus seems unable to penetrate. Not infrequently these same burls are infected by *Nectria cinnabarina* Fr. In one interesting case two other fungi, *Stereum hirsutum* Willd, and *Polystictus hirsutus* Schrad., had become established in the tissues of the burl already housing the three more serious fungi. It is interesting to note that the mycelium of any one of the wood destroying fungi did not encroach upon tissues already infected by another.

The presence of the Nectria in the cankers of Plowrightia on *Prunus demissa* Walp. and *P. emarginata* Walp. and in a lesser degree on *Amelanchier alnifolia* Nutt. is of quite common occurrence throughout the range of these species in Montana and Idaho. In the Bitterroot Valley *Nectria cinnabarina* or probably a variety of this species is very destructive to *Sambucus glauca*.

*Plowrightia morbosa* is quite widely distributed in the Northwest and does immense damage to wild species of *Prunus*. In the laboratory collection at Missoula is a specimen on *Prunus subcordata* Benth. from southern Oregon. In the vicinity of Salmon Arm, British Colombia, the writer made a collection of the fungus from what is presumably *Prunus pennsylvanica* L. Other collections at hand are on *Prunus emarginata*, Metaline Falls, Washington, *Prunus demissa*, Coeur d'Alene Mountains, Idaho, and on several varieties of cultivated plums and cherries from various regions of the Northwest.

JAMES R. WEIR

# LITERATURE ON AMERICAN PLANT DISEASES

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All authors are urged to coöperate in making this list complete by sending their separates and by making corrections and additions, and especially by calling attention to meritorious articles published outside of regular journals. Reprints or correspondence should be addressed to Miss E. R. Oberly, Librarian, Bureau of Plant Industry, U. S. Dept. Agric., Washington, D. C.

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